# Investigation of endothelin-1 contribution to pulmonary arterial hypertension by proteomic mass spectrometry (12/20 words limit)

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## Introduction (119/120 words limit):

Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary vascular resistance and remodeling. It is found that increased vascular smooth muscle cell (VSMC) hypertrophy, migration and proliferation are among the key events that contribute to vascular remodeling. Endothelin-1 (ET-1), a potential vasoconstrictor produced by vascular endothelial cells, activates several signal transduction pathways linked to remodeling in several cell types in cardiac tissues and cardiomyocytes. Here we focus the investigation of ET-1 contribution to the development of PAH, by analyzing the protein expressions in pulmonary arterial smooth muscle cells (PASMC) from normal and PAH subjects with/without ET-1 stimulation using proteomic mass spectrometry. Our results provide protein candidates important for understanding the mechanism of ET-1 on the PAH pathogenesis.

## Methods (119/120 words limit):

PASMC were obtained during lung transplant from a healthy donor and a PAH patient, and were incubated with (treated group) or without (control group) 100 nM ET-1 in serum free medium for 24 h. Fifty μg of reduced/alkylated PASMC lysate was digested with trypsin and the tryptic peptides were desalted with an OMIX C18 tip. Eluted peptides were analyzed in triplicate with an Acuity nanoflow UPLC (Waters) coupled with a TriVersa NanoMate (Advion)-electrospray ionization source to an LTQ-Orbitrap mass spectrometer (Thermo Fisher). Label-free quantitation was performed with Progenesis. Peptide/protein identifications were searched against SwissProt human database by Mascot. Relative protein abundances were calculated based on peptide ion intensity values, and biological signaling pathways were analyzed with Ingenuity IPA software.

## Preliminary data (294/300 words limit):

Label-free quantitation LC-MS/MS analysis was performed on PASMC from one control and one PAH patient, with/without ET-1 treatment. With the stringent filtration criteria applied (mass error: 5 ppm, MS1; 0.5 Da, MS2; ion score ≥20; p-value < 0.05; ≥ 3 unique peptides), 376 proteins were identified from the 4 samples. Seven proteins including tropomyosin α4 chain (TPM4), chloride intracellular channel proteins1/4 (CLIC1/CLIC4), four and a half LIM domains protein 1(FHL1), were previously associated with PAH in lung tissue. IPA analysis showed these proteins may be involved in pathways such as EIF2, EIF4 and p70S6K, mTOR, RhOA and integrin signaling, and protein ubiquitination. They can contribute to growth and hypertrophy of PASMC, muscle contraction and cell death.

Pairwise comparisons indicated that ET-1 increased the abundance of all proteins identified in healthy control PASMC, but 20 up-regulated and 12 down-regulated proteins (absolute fold change (FC) ≥2) were observed in patient PASMC. Heme oxygenase 1 (HMOX1) (FC 4.87) has been associated with mTOR, phospholipase C and endothelin-1 signaling and phospholipid

degradation, while NAD (PH) dehydrogenase 1 (NQO 1) (FC 2.24), might be involved in hypoxia signaling in the cardiovascular system and related to the NRF2-mediated oxidative stress response. For most of these 32 up/down-regulated proteins in PAH, ET-1 stimulation increased their expressions to an extent similar to the control, with the exception that restoration was impeded for 3 proteins: collagen alpha-3(VI) chain, transforming growth factor-β-induced protein ig-h3 and protein-glutamine γ-glutamyltransferase 2; all three proteins are reported to be important in cell binding and conjugation. Thus protein expression changes in PASMC induced by ET-1 may involve multiple signaling pathways. Further analysis with more patients' PASMC and biological tests are underway. This research is supported by NIH NCRR P41 RR01088, S10 RR020946 and NHLBI N01 HV00239 and R01 HL025776.

**Novel aspect (17/20 words limit)**: Label-free quantitation LC-MS/MS analysis was used to investigate endothelin-1 involvement in signaling pathways in pulmonary arterial hypertension.