Title: VE-Cadherin downregulation may be a feature of endothelial transdifferentiation in monocrotaline-induced Pulmonary Hypertension

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Background: Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by a sustained elevation of pulmonary arterial pressure. Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor peptide, suggested to contribute to pulmonary hypertension pathogenesis. Vascular endothelial cadherin (VE-cadherin) is found in the adherens junctions of the endothelial cells and is a protein essential for embryonic angiogenesis and vascular maintenance. Endothelial-to-mesenchymal transition (EnMT), the process through which endothelial cells gain expression of myofibroblast-like characteristics, is shown to be important during cardiovascular development and appears to play a role in several vascular pathologies. Based on the above, we sought to investigate whether there is a potential role of endothelial transdifferentiation in the monocrotaline (MCT) rat pulmonary hypertension (PH) model.

Methods: MCT-induced PH was generated in rats after a single intraperitoneal (i.p.) injection of monocrotaline (60 mg/Kg). The control group received an i.p. injection of saline. Animals were sacrificed at 1, 15 and 30 days post injections. PH development was validated by calculating the right ventricle/(left ventricle+septum) weight ratio. Lung tissues were processed for immunostaining, immunoblotting and RNA isolation. Apart from the animal model study, a series of in vitro experiments in rat lung microvascular endothelial cells (RLMVECs) were performed. Cells were cultured in standard conditions for 2-3 passages and after exposure to ET-1, lysates and total RNA were isolated.

Results: Early reduction of VE-Cadherin mRNA and protein levels was found, concomitantly with apoptosis and proliferation of lung cells. These findings could possibly indicate a phenotypic shift, when associated with the observation that the mesenchymal markers vimentin and α-smooth muscle actin (α-SMA) were over-expressed following MCT challenge. The transcription factors Snail and Slug (previously found expressed in other EnMT models) were upregulated in the lungs of our MCT-treated rats. NF-κB and endothelial nitric oxide synthase (eNOS) pathways were activated, and reactive nitrogen species were detected. In accordance with the in vivo experiments, ET-1 promoted loss of VE-Cadherin and concomitant increase of vimentin in RLMVECs. Snail expression from RLMVECs was upregulated upon ET-1 stimulation and the NO pathway was found to be involved in this process.

Conclusions: Our data suggest that aberrant pulmonary endothelial cells in the rat monocrotaline model may acquire characteristics of vascular smooth muscle cells and myofibroblasts. As the diseased endothelium can be a factor perpetuating vascular lesions in PAH, comprehending the extent of this phenomenon may help in better understanding PAH pathogenesis.

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