

# INVOLVEMENT OF PLATELET-DERIVED GROWTH FACTOR AND THROMBIN IN VASCULAR REMODELING IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION

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**Objective:** The pathogenic mechanisms involved in proximal and distal pulmonary arterial remodeling in patients with chronic thromboembolic pulmonary hypertension (CTEPH) remain unclear, although cellular and molecular mechanisms of idiopathic pulmonary arterial hypertension have been extensively studied during the last decades. Platelet-derived growth factor (PDGF) and thrombin are known as important stimuli for proliferation and migration of pulmonary artery smooth muscle cells (PASMC). This study aimed at investigating whether PDGF and thrombin are involved in pulmonary vascular remodeling in CTEPH.

**Methods:** The endarterectomized tissues from patients with CTEPH were used to isolate and prepare pulmonary vascular smooth muscle cells with the written informed consent and the IRB approval. The pulmonary vascular segments of the endarterectomized tissues were digested with collagenase and elastase to isolate vascular cells (CTEPH cells). The cells were resuspended and plated onto Petri dishes in smooth muscle growth media (SMGM). Normal human PASMC were cultured under the same condition as were CTEPH cells. When reached 80% confluence, the cells were growth-arrested and then PDGF or thrombin (along with rapamycin or Akt inhibitor) was added to the media. Western blot analysis was performed to determine phosphorylation of signaling proteins in Akt and ERK pathways. Intracellular Ca<sup>2+</sup> imaging system was used to measure changes in cytosolic Ca<sup>2+</sup> with fura-2.

**Results:** In CTEPH cells and normal PASMC, PDGF and thrombin induced marked phosphorylation of multiple signaling proteins in the Akt/mTOR and ERK pathways, and enhancement of store-operated Ca<sup>2+</sup> entry (SOCE). Chronic treatment with rapamycin or Akt inhibitor significantly attenuated the PDGF-mediated augmentation of SOCE and thrombin-mediated Ca<sup>2+</sup> release in these cells.

**Conclusions:** The data suggest that both PDGF and thrombin play an important role in pulmonary vascular remodeling in CTEPH. PDGF and thrombin cause CTEPH cell proliferation by enhancing SOCE through activation of the Akt/mTOR pathway. Modifying this pathway may have potential to develop novel therapeutic approach for CTEPH patients with persistent post-operative pulmonary hypertension.