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CELLULAR MECHANISMS OF KV1.5 DOWNREGULATION IN IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION-PULMONARY ARTERY SMOOTH MUSCLE CELLS

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INTRODUCTION: Idiopathic pulmonary arterial hypertension (IPAH) is a serious disease that is ultimately fatal. It is known that pulmonary artery smooth muscle cells (PASMC) from patients with IPAH (IPAH-PASMC) grow faster than normal PASMC due to a disruption between cell proliferation and apoptosis, creating an apoptosis-resistant phenotype. The decreased amplitude of whole cell Kv currents and the downregulated mRNA/protein expression of potassium channels, including Kv1.5, is related to the progression of pulmonary hypertension and linked to attenuated apoptosis. Cell shrinkage (or volume loss) is the hallmark of apoptotic cells. It has been reported that decreased intracellular potassium concentration is involved in apoptotic volume decrease regulation. Efflux of potassium through Kv channels plays a role in initiating apoptosis. Low concentration of intracellular potassium is also necessary for activation of caspases and nucleases that cause DNA fragmentation. Decreased activity of Kv channels in PASMCs from patients with IPAH and animals with chronic hypoxia-induced pulmonary hypertension inhibits apoptotic volume decrease and attenuates apoptosis. FOXM1 is an important transcription factor that regulates cell cycle progression and may induce transcriptional regulation of Kv channels, but may not have direct functional effect on Kv channel activity. We hypothesized that activation of FOXM1 leads to decreased gene expression of Kv1.5 in IPAH-PASMC.

METHODS: Human PASMC from normal subjects and IPAH-PASMC were cultured. Kv1.5 gene expression for both cell types was determined by semi-quantitative RT-PCR. Normal PASMC and IPAH-PASMC

were treated with either Staurosporin or vehicle (DMSO). Apoptosis level was quantified using TUNEL assay in IPAH-PASMC compared to normal PASMC.

RESULTS: RT-PCR showed a downregulation of gene expression of Kv1.5 in IPAH-PASMC compared to normal PASMC. TUNEL assay revealed a smaller percentage of apoptosis in IPAH-PASMC than in normal PASMC when treated with Staurosporin.

DISCUSSION: Based on preliminary semi-quantitative RT-PCR results, Kv1.5 gene expression is downregulated in IPAH-PASMC as expected. Previous studies have shown that a decrease in Kv1.5 expression leads to inhibition of caspases and a decrease in apoptosis in IPAH-PASMC. TUNEL assay showed a smaller percentage of apoptotic IPAH-PASMC than normal PASMC when treated with Staurosporin. Future experiments include performing Western blot and real-time PCR to determine if the expression of Kv1.5 is affected by inhibition of FOXM1 via pharmacological and small interfering RNA in PASMC. An additional experiment would be to perform TUNEL assay comparing normal PASMC, FOXM1 inhibited normal PASMC, and FOXM1 inhibited IPAH-PASMC. We would expect to see an increase in apoptosis in the FOXM1 inhibited cells. As there is currently no cure for IPAH, there is a need to develop alternative methods of treatment. This research may eventually contribute to a treatment that reduces PASMC proliferation and increases PASMC susceptibility to apoptosis.