Knockdown of bone morphogenic protein receptor–II by siRNA increases in vitro endothelin-1 synthesis by human pulmonary microvascular endothelial cells

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Introduction: Idiopathic pulmonary arterial hypertension (IPAH) is a rare disorder that causes cellular narrowing of the pulmonary microvasculature vasculature, resulting in right heart failure and death. Endothelial dysfunction is a hallmark, with altered levels of endothelial-derived mediators, including endothelin-1 (ET-1). It is unknown what triggers the increased ET-1 levels. A heritable form of IPAH has been described, and is most frequently caused by mutations coding for bone morphogenic protein receptor–II (BMPR2). The effects of BMPR2 dysfunction or decreased levels on endothelin synthesis are unknown. We hypothesized that decreasing BMPR2 levels would increase ET-1 levels.

Methods: Human pulmonary microvascular endothelial cells (HMVEC-LBl) were obtained from Lonza and grown to 75% confluency in EGM-2MV medium on 24 well plates. siRNA to human BMPR2 and scrambled control (Dharmacon) was reconstituted in RNA reconstitution solution. The HMVEC-LBL were transfected with Dharmafect 4 (Dharmacon) containing siRNA or scrambled control RNA for 6 hours at 37^{o}C. They were then incubated in complete medium for 72 hours then serum starved overnight. The medium was then replaced with fresh endothelial basal medium (EBM, Lonza) for 8 hours, and the supernatant was collected for ET-1 measurement by ELISA (Enzo). The cells were then lysed for total protein content. ET-1 levels were normalized to total protein. N=12 wells were used per condition. Group comparisons were made using Tukey-Kramer analysis (α=0.02).

Results: In several experiments, BMPR2 knockdown increased mean normalized ET-1 levels in the medium by 30-50% as compared to scrambled control (0.418 ± 0.09 SD pg ET-1/ µg protein versus 0.273 ± 0.06). There was no effect on cellular protein levels per well.

Conclusion: Low BMPR2 levels are associated with increased ET-1 levels in culture. The BMPR2 mutations seen in heritable PAH may be contributing to the increased ET-1 seen in human.