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Profile of Pulmonary Smooth Muscle Cells and Their Response to Blockade and Stimulations

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INTRODUCTION: The tone of the pulmonary arteries is the summation of the activity of each smooth muscle cell (SMC) within a vessel wall and its interaction with the endothelial cells and extracellular matrix (including collagen). There are reported phenotypic differences between SMC in the inner & outer layers of pulmonary artery walls[1]. To tissue engineer a blood vessel a basic understanding of differences between inner and outer SMC's in terms of attachment and contraction of 3D matrix is essential. We hypothesised that they will differ in their ability to contract a 3D collagen gel. Using a Culture Force Monitor (CFM) we sought to: (1) quantify the contractile ability of SMC derived from inner and outer normal and hypoxic arteries, harvested from piglet models, over 24 hours, (2) test the effect of cytoskeletal blockade on force retention in collagen gels and (3) quantify cellular contractile response to agonist or antagonist stimulation.

METHODS: Piglets were exposed to hypoxia (50KPa) for 3-14 days then sacrificed at day 14. Large intrapulmonary arteries were dissected and SMC derived from inner & outer layers were then cultured in DMEM/F12 medium with 10% FCS. 5 ml rectangular Collagen gels2 (rat tail collagen type I, 10x minimal essential medium, sodium hydroxide) were prepared in a sterilised silicone polymer mould and seeded with 5 million cells (passage 3-6). The gel was allowed to set with 2 A-frames (layered polyethylene mesh with a stainless steel frame) on either side and then suspended in DMEM with 10% FCS. One A-frame was connected to a fixed point in the CFM while the other is connected to a transducer. Real time contractile force generated (1 per second) and cellular response were recorded over 24 hours (at 37 ^{o}C, 5% CO^{2}).

RESULTS: Normal Outer SMC's generated highest peak force (mean 450 dynes). Hypoxic outer SMC's showed a significant ($p<0.005$) decrease in their peak force generation (mean 190 dynes). Normal Inner SMC's generated lower peak force compared to normal outer SMC's (mean 320 dynes to 450 dynes (approximately 30% decrease). Hypoxic inner SMC's also demonstrated a slight decrease in their contractile ability (mean 290 dynes compared to 390 dynes (approximately 25% decrease). Using agonist U46619 we demonstrated a significant increase in the contraction force generated, while an antagonist SNP, a SMC relaxant, resulted in significant loss of the contraction ability of these cells.

DISCUSSION AND CONCLUSIONS: We demonstrated that SMC's derived from the normal outer layer of pulmonary vessels generate a significantly greater peak force compared to SMC's from the inner layers of these vessels. However on exposure to hypoxia, the outer SMCs, had a significant reduction in their contraction capabilities. Hypoxia had no effect on contraction of inner wall SMC's by comparison. The cells also showed an appropriate response when stimulated with known agonists and antagonists. This has previously been demonstrated in 2D or whole tissue and validates the 3D model used in our study. Hypoxia induced hypertension causes the outer SMCs to lose their tone maintaining capabilities in vivo, a feature of pulmonary hypertensive vessels. They also lay down significant matrix, which increase vessel wall stiffness. Our findings clearly demonstrate significant differences in peak force generation and contraction between SMC's resident in the inner and outer walls of pulmonary arteries. The pulmonary arteries need to be structurally and mechanically stable for function. Our findings have implications for functional Tissue Engineering of blood vessel's as different layers of SMC's will need to be engineered or sourced to generated specific range of force and contraction within a mechanically stable construct.

REFERENCES: [1] S.M. Hall, A. Hislop, C. Pierce et al (2000) prenatal origins of human intrapulmonary arteries. *Am J Respir Cell Mol Bio* **23**:194-203.