

REFERENCE

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THE EFFECT OF HYPOXIC MEDIATED PRO-MITOGENIC SIGNALLING ASSOCIATED WITH VEIN GRAFT FAILURE AND VASCULAR REMODELLING

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Tissue hypoxia is known to contribute to the hyperproliferative pathogenesis underlying vein graft stenosis with loss of luminal patency. Hypoxia has been reported to modulate PLC- γ leading to the activation of PKC dependent-cell proliferation through mitogen activated protein kinases (MAPK) signalling pathways. Additionally, hypoxia is positively correlated with the pro-mitogenic HIF-1 α derived signalling pathway. This project aims to investigate the potential interaction of HIF-1 α and PLC- γ in hypoxic mediated pro-mitogenic signalling associated with vein graft remodelling.

Vascular smooth muscle cells (VSMC) were cultured in hypoxic conditions (3%–10%) and compared with normoxic culture. VSMC proliferation was assessed through ³ hour thymidine incorporation assays, mRNA expression levels were measured using RT-PCR and MAPK signalling was evaluated by immunoblotting. Conditioned media experiments from VSMC and endothelial cells were performed along with exosome isolations from both cell types under experimental conditions.

Results confirmed a positive correlation between hypoxia and cell proliferation; cells cultured under 3% and 10% oxygen increased cell proliferation by 2.1 and 1.4 fold respectively. The PLC- γ inhibitor U73122 (1 μ M) significantly reduced VSMC proliferation under normoxia however had no inhibitory effect in hypoxic culture. Experiments also showed hypoxia to downregulate mRNA expression levels of PLC- γ , by 49.9%.

Conditioned media from 3% hypoxic cultured endothelial cells increased VSMC proliferation by 5.8 fold at 24 hours. Initial experiments have confirmed a hypoxic-dependent-increase in exosomal trafficking where the measured exosomal yield from VSMC cultured under 3% hypoxia increased by 30% when compared with normoxic cultures.

Moderate hypoxia significantly drives VSMC proliferation in comparison to mild hypoxia. These results raise questions over the association with PLC- γ and hypoxia-dependent pro-mitogenesis of VSMCs. This observation is backed up by the absence of hypoxic-dependent phosphorylation of the MAPKs. Of note, initial experiments have identified hypoxic culture modulates a pro-mitogenic endothelial-derived transferable factor(s) and increase in exosomal trafficking.

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INVESTIGATING CALCIFIC AORTIC VALVE DISEASE USING NOVEL IMMORTALISED SHEEP AND RAT VALVE INTERSTITIAL CELL LINES

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Calcific aortic valve disease (CAVD) involves progressive valve leaflet thickening and severe calcification, resulting in impaired leaflet motion. Although much research has advanced our knowledge of this disease, the mechanisms underlying the initiation and progression of CAVD are still unclear, necessitating further studies to elucidate the underpinning processes in the early stages of this disorder.

Our present study aimed to (i) generate and (ii) evaluate the calcification potential of immortalised cell lines derived from sheep and rat aortic valve interstitial cells (VICs). We show that both sheep (SAVIC) and rat (RAVIC) cell lines expressed markers of VICs (vimentin and α -SMA). We also established that sheep VIC (SAVIC) calcification can be induced in the presence of increased calcium only (2.7 mM; 1.9 fold; $p < 0.001$), with a synergistic effect of calcium and phosphate on VIC calcification noted at 2.7 mM and 2.0 mM, respectively (22.2 fold; $p < 0.001$). Significant increases in mRNA expression of key genes associated with valve calcification were observed (RUNX2 and PiT1) when SAVICs were cultured under increasing calcium conditions. Contrastingly, the mRNA level of a potential calcification inhibitor (MGP) decreased under these conditions. Interestingly, we found very little alkaline phosphatase (ALPL) expression in these cells. Comparable data were observed in the RAVIC studies. Furthermore, SAVIC calcification levels were significantly reduced in the presence of known inhibitors of calcification, pyrophosphate (PPi; 1.7 fold; $p < 0.01$) and etidronate (3.2 fold; $p < 0.01$).

In conclusion, the use of immortalised sheep and rat VICs can provide a reliable model system to investigate aortic valve calcification *in vitro*, which will assist in our understanding of this pathophysiological process

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FEMORAL ARTERY LIGATION INDUCES RAPID ARTERIOGENESIS THAT CORRELATES WITH RECOVERY OF PERFUSION IN THE FOOT

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Background and hypothesis Reperfusion following acute hindlimb ischaemia is mediated by both arteriogenesis (collateral growth) and angiogenesis. According to Poiseuille's Law, the arterial ability to conduct blood is proportional to the 4th order of its diameter. This suggests collateral arteries with a relatively large diameter are better suited to conduct blood than smaller vessels. We hypothesise that arteriogenic remodelling of collateral vessels has a greater role than angiogenesis in restoring blood supply to the ischaemic hindlimb.

Methods Acute hindlimb ischaemia was induced in 12-week-old male C57BL/6J mice by unilateral femoral artery ligation. Reperfusion in the foot pad was monitored by laser Doppler immediately before and after femoral artery ligation and subsequently on days 1, 3 and 7 (n=3 for each time point). Vascular resin casts plus optical projection tomography (OPT) were used to illustrate the arterial network before and after ischaemia.

Results Blood perfusion in the foot pad dropped by ~90% immediately following ligation but exhibited a 40% recovery over the next 7 days. This restoration of flow correlated with