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Insulin-Like Growth Factor Binding Protein-2: A Putative Agent for Therapeutic Angiogenesis Acting via Its Arginine-Glycine-Aspartic Acid (RGD) Domain

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Therapeutic angiogenesis is under investigation to restore tissue perfusion in ischaemic heart disease and peripheral arterial disease. Insulin-like growth factor binding protein-2 (IGFBP-2) has been attributed with pro-angiogenic activity but the molecular mechanisms have not been established. Structurally, IGFBP-2 possesses insulin-like growth factor (IGF) binding-, integrin recognition- (Arginine-Glycine-Aspartic Acid (RGD)) and heparin binding- sites and a nuclear localisation signal which may be implicated in its cellular actions.

We hypothesise that IGFBP-2 can be exploited to promote therapeutic angiogenesis through cellular actions mediated by one or more of its structural domains.

Restoration of limb perfusion was quantified by laser Doppler imaging following hind limb ischemia in mice expressing human IGFBP-2. Mechanistic studies were carried out in cultured human umbilical vein endothelial cells (HUVEC). Functional angiogenic responses were explored with tube formation and cell adhesion assays. Wild type IGFBP-2 (^{WT}IGFBP-2) and recombinant IGFBP-2 containing a non-functional IGF binding site (IGFΔ) or mutated RGD domain (RGDΔ) were generated to identify the structural domain responsible for the pro-angiogenic signalling mechanisms.

Transgenic expression of hIGFBP-2 in mice significantly enhanced restoration of blood flow to the limb at day 7 compared to WT littermates ($p<0.05$). Both ^{WT}IGFBP-2 and $^{IGF\Delta}$IGFBP-2, significantly increased tube formation in HUVEC. Interestingly, $^{RGD\Delta}$IGFBP-2 stimulated tube formation was greatly decreased compared to ^{WT}IGFBP-2 ($p<0.05$). ^{WT}IGFBP-2 increased HUVEC adhesion to fibronectin (1.6 fold); however this effect was absent with $^{RGD\Delta}$IGFBP-2. Stimulation of endothelial cells with ^{WT}IGFBP-2 induced Akt phosphorylation (1.3 fold). $^{RGD\Delta}$IGFBP-2 did not significantly activate Akt. Neither ^{WT}IGFBP-2 nor the mutants induced the canonical angiogenic signalling factors, such as eNOS, FAK or MAPK phosphorylation.

IGFBP-2 promotes angiogenesis in vitro and in vivo, supporting its further investigation for therapeutic angiogenesis in ischaemic disorders. For the first time, we demonstrate that the RGD domain of IGFBP-2 appears to play a critical role in angiogenic activity.