RNA Transport in Angiogenesis: Deciphering Mechanisms of RNA Localisation to Understand Blood Vessel Growth

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Background: Angiogenesis, the formation of new blood vessels from pre-existing vasculature, involves the coordinated migration of endothelial cells, led by highly polarised tip cells. Polarisation of migrating tip cells involves RNA localisation, the asymmetric distribution of RNAs within the cells. Mechanisms that regulate endothelial cell migration during angiogenesis are well understood, however research into the role of RNA-interacting proteins in RNA localisation required for this endothelial cell migration is lacking.

Preliminary data identified around 150 RNA-bound proteins at the front of migrating human umbilical vein endothelial cells (HUVECs), from which AKAP12 and YB1 were selected for investigation. AKAP12, an A-Kinase-Anchoring protein implicated in angiogenesis and cytoskeleton dynamics has not been reported as RNA interacting. YB1, a Y-box binding protein, is an RNA-binding protein (RBP) implicated in many cellular functions and the RNA lifecycle, however research into its role in angiogenesis has been minimal. Therefore, this project aims to address two important questions; what RNAs do they regulate at the cell front and what do these interactions mean for tip cell migration during angiogenesis?

Methods: UV-RIP was used to immunoprecipitate AKAP12 from HUVECs and immunoprecipitates were subjected to RNA-sequencing. Gene Ontology analysis categorised targets based on their molecular function. The pEGFP-YB1 plasmid was generated by subcloning a YB1 fragment from pDESTmycYBX1 into vector pEGFP-C1 and deletion mutations were introduced into plasmid EGFP-N3 α-SSeCKS. Downregulation of AKAP12 and YB1 in HUVECs was performed via siRNA-mediated knockdown. HUVEC-HPF co-cultures were used to form endothelial networks under VEGF treatments of increasing concentrations. Networks were then stained via immunofluorescence and imaged using a widefield microscope to allow analysis of network variables using ImageJ.

Results: Preliminary data identified AKAP12 as being RNA-bound at the front of migrating endothelial cells. RIP-seq was able to confirm this previously unreported function of AKAP12 as an RNA-interacting protein and provided insight into its targets. Evidence of interactions between AKAP12 and its targets was investigated by qPCR. Computational analysis using YB1 RIP-seq data found in literature revealed targets that are significantly enriched in protrusions of migrating endothelial cells.

A GFP-tagged YB1 plasmid was successfully cloned and will be used to elucidate the localisation of YB1 in live cells. AKAP12 mutants, lacking domains responsible for targeting AKAP12 to the cell periphery, were produced to evaluate the subsequent impact on both the translation and localisation of its target mRNAs.

Endothelial networks were optimised through HUVEC and HPF co-cultures treated with VEGF, where a concentration of 10 ng/µL VEGF was identified as producing the most complex networks. Co-cultures with AKAP12 or YB1 knockdown HUVECs investigated the effects that these RBPs have on endothelial network formation, with analysis comparing the total number of vessels and the average number of branches per area of the networks.

Conclusions: These results encourage the exploration of how AKAP12 and YB1 regulate their target mRNAs, followed by investigation of how disruption of interactions between AKAP12/YB1 and their target RNAs may affect the localisation or translation of the RNAs. *In vitro* angiogenesis assays will be used to investigate how downregulation of these RBPs affects interacting RNAs and how, in turn, that may modulate endothelial cell migration during angiogenesis.