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THE RNA-BINDING PROTEIN QUAKING-7 IS A TARGETABLE REGULATOR OF DIABETIC VASCULOPATHY

Endothelial cell (EC) health is intricately linked with vascular homeostasis, dysfunctional ECs are therefore key mediators of vascular disease onset and progression, the leading cause of mortality worldwide. Moreover, individuals with diabetes have up to a fourfold increased risk of developing vascular diseases. Despite the high morbidity, mortality and economic burden associated with vascular diseases, the mechanisms behind diabetic vascular dysfunction remain predominantly unclear, limiting treatment strategies. Advancements in regenerative medicine, largely the use of induced pluripotent stem cell (iPSC) technologies, have provided powerful tools to study vascular health and deepen our understanding of endothelial cell dysfunction. Importantly, the derivation of endothelial cells from human iPSCs (iPS-ECs) has revealed a role for RNA binding proteins (RBPs) within the vascular system, including uncovering their roles as key mediators of disease development.

Recently, we discovered the RBP QKI-7 to be significantly upregulated in diabetic endothelial cells, due to a dysregulation of RNA splicing factors CUG-BP and hnRNPM. QKI-7 overexpression greatly impaired endothelial cell barrier, compromised angiogenesis and enhanced monocyte adhesion. Furthermore, knockdown of QKI-7 in vivo, using a hindlimb ischemia diabetic mouse model, resulted in significant reperfusion and blood flow recovery. Elucidation of QKI-7 as a regulator of endothelial health therefore has substantial potential to reveal novel therapeutic strategies for diabetic individuals. To evaluate this hypothesis and identify enriched RNAs as a result of QKI-7 overexpression, RNA-immunoprecipitation Sequencing was performed on diabetic and non-diabetic iPS-ECs. Differential expression analysis was performed using the R package DESeq2, followed by standard visualization and interpretation using other R packages including EnhancedVolcano and Pheatmap. Comparative enriched RNAs were filtered according to the absolute log2 fold change greater than +1 and corrected p-value threshold of <0.05. Of a total of 2129 differentially enriched RNAs, we isolated 42 RNAs (17 Coding and 25 Non-Coding) to be statistically significantly enriched by QKI-7. To allow for the elucidation of the potential pathogenic mechanisms, Ingenuity Pathways Analysis was performed to highlight canonical pathways affected by QKI-7 overexpression. The enriched RNAs were revealed to have fundamental roles in both vascular development and vascular disease onset networks as well as be affiliated with 48 pathways connected to vasculature health including processes such as vasculogenesis, angiogenesis, and endothelial cell growth and proliferation. Interestingly, many were shown to have an affiliation with diabetes and diabetic complications also. Overexpression of QKI-7 in ECs, as well as analysis of diabetic iPS-ECs, confirmed QKI-7 to significantly regulate the expression of 20 of these enriched RNAs. Moreover, using the platform RBPmap, numerous binding sites of QKI-7 within these enriched RNAs were identified, emphasising the role of QKI-7 as a key regulator of diabetic endothelial dysfunction.

Through direct gene regulation, the RBP QKI-7 is able to bind and mediate a vast network of endothelial cell dysfunction, contributing to the progression of vascular disease in diabetic individuals. As demonstrated in vivo, manipulation of QKI-7 therefore represents a promising strategy for the treatment of diabetic vascular complications.