Role of cFOS in mechanosensitive transcriptional regulation in diabetes associated atherosclerosis

A. Khan[1], M. Lee[2], A. Watson[1], S. Maxwell[1], M. Cooper[1], A. Murphy[2], K. Jandeleit-Dahm[1]

[1]Monash University, Melbourne, Australia; [2]Baker Heart and Diabetes Institute, Melbourne, Australia

Funding Acknowledgement: Type of funding sources: Foundation. Main funding source(s): National Heart Foundation of Australia

Atherosclerosis, as manifested clinically by myocardial infarction, stroke and peripheral vascular disease, is a major contributor to cardiovascular disease, the leading cause of death in patients with diabetes. The lipid-laden plaque development within the arterial vessel wall is a progressive process initiated with endothelial cell activation and monocyte adhesion. These cellular events occur primarily at the regions of blood vessels exposed to turbulent blood flow (TBF) and low shear stress such as vascular bends and bifurcations. Exposure of the vascular cells to chronic hyperglycaemia and TBF induces a proatherogenic transcriptional profile. Studies have shown that shear stress regulates vascular pathophysiology via differential regulation of transcription factors (TFs) such as KLF4, EGR1 and AP-1, hence named as mechanosensitive TFs. AP-1 is a heterodimer composed of FOS, Jun and ATF family of TFs. Studies have shown that it is activated by low shear stress in cultured endothelial cells. Increasing evidence supports the vital role of AP-1 family members in inflammation and diabetes-induced myocardial dysfunction. However, gene targets and the mechanisms underlying hyperglycemia-induced activation of AP-1 transcription factor cFOS in vascular regions exposed to TBF are not known.Although a novel approach not previously studied in diabetes associated atherosclerosis, we used a single cell RNA sequencing (scRNA-seq) approach to identify endothelial cells from TBF regions of aorta. Diabetes was induced with streptozotocin (STZ) in Apoe–/– mice and followed for 10 weeks. Cells from digested aortae of control and diabetic mice were subjected to scRNA-seq using 10X Genomics system and Illumina Nova-seq 6000. Unsupervised graph based clustering grouped cells into fourteen cell clusters with similar gene expression profile. We applied a list of mechanosensitive gene markers including EGR1, cFOS, Junb and ICAM1 in scRNA-seq analysis to identify endothelial cells from TBF regions of aorta. This approach identified atheroprone endothelial cells exposed to persistent TBF that showed a distinct transcriptional profile with more than six hundred genes differentially expressed. Importantly, cFOS was the most significantly upregulated gene in endothelial cells exposed to TBF. We next generated adiabetes associated transcriptional signature unique to endothelial cells exposed to TBF as compared to all other cell types in the aorta. We identified several genes in endothelial cells exposed to TBF and hyperglycaemia uniquely dysregulated in diabetic Apoe–/– mice as compared to control mice (cut off = FDR$<$0.05, fold change at least 2-fold). Gene set enrichment analysis identified "fluid shear stress and atherosclerosis" as most significantly dysregulated pathway in endothelial cells. These novel findings indicate that AP-1 TF subunit cFOS is a potential therapeutic target in diabetes associated atherosclerosis that warrant further experimental exploration.