Loss of heparan sulphate expression causes the endothelial-to-mesenchymal transition and cell dysfunction in coronary microvascular endothelial cells

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Aim: The endothelial glycocalyx (eGlx) is a brush-like sugar layer lining the luminal side of vascular endothelial cells. It is therefore a vital regulator of vascular permeability. We have recently shown that damage to the coronary microvascular endothelial (CMVEC) glycocalyx contributes to the pathology of diabetic cardiomyopathy. Restoration of the glycocalyx improved diastolic function and reduced cardiac oedema (1). The eGlx is a heterogeneous structure yet most studies have examined crude measures including volume or depth and hence understanding of the role of individual components is limited. Heparan sulfate (HS) is an important component in eGlx and Ext1 is the rate-limiting enzyme in HS biosynthesis. The HS-degrading enzyme heparanase is increased and HS is damaged in diabetes. We therefore hypothesise that HS is a key component of the CMVEC glycocalyx and represents a potential therapeutic target for diabetic cardiomyopathy.

Methods: The importance of HS to the CMVEC glycocalyx structure and function were characterised by deleting HS using Ext1 shRNA to knock down the Ext1 expression. HS removal was confirmed by RT-qPCR, Western blotting, and immunofluorescence. CMVEC functional changes were determined by trans-endothelial permeability to albumin assay and analysis of cell response to laminar shear stress, indicated by the ability to upregulate KLF2 mRNA expression.

Results: In cells transduced with Ext1 shRNA, Ext1 mRNA expression was reduced by about 60%, which corresponded to a nearly 50% reduction of glycocalyx HS. HS removal did not affect another glycocalyx component Syndecan 4 expression. Reduced HS expression was associated with the elevated trans-endothelial passage of albumin (8.30 ± 0.24 vs. 5.66 ± 0.22μg/ml, n=6, ***p<0.001), indicating increased cell permeability. Moreover, the ability of CMVEC to respond to laminar shear stress was damaged, evidenced by significant impairment in cell ability to upregulate KLF2 mRNA expression (3.12 ± 0.43 vs. 8.61 ± 0.28-fold relative to controls, n=3, ***p<0.001), and to align cellular actin in response to laminar shear stress. These functional changes are likely due to CMVEC going through the endothelial-to-mesenchymal transition (EndMT) caused by reduced HS expression. CMVEC transduced with Ext1 shRNA showed decreased endothelial properties, e.g., cobblestone-like morphology and the expression of characteristic surface endothelial marker VE-cadherin, and acquired mesenchymal features, e.g., fibroblast-like cell morphology.

Conclusions: The results prove the importance of HS to CMVEC function. Loss of glycocalyx HS causes elevated cell permeability and reduced cell ability to respond to laminar shear stress. This is probably due to CMVEC going through EndMT after glycocalyx HS loss. Thus, glycocalyx HS is a key potential therapeutic target to protect CMVEC function.

References: (1). Qiu Y et al. Diabetologia. 2022 May;65(5):879-894.