S22: Glycocalyx – Its Role in Disease

S22-1 Role of the Glycocalyx in Atheroprotective vs. Atheropermissive Endothelium Function

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Atherosclerosis occurs at vessel sites exposed to complex flow patterns, which damage endothelium. Proper endothelium function relies on the protective glycocalyx (GCX), which is shed in disease. Replacing it may heal ECs and slow disease progression. We studied cultured ECs and performed mice experiments, to examine endothelium in healthy and disruptive flow conditions. Immunocytochemical studies verified spatial variations in EC GCX composition. We correlated GCX composition to EC functions including vasoregulation, communication, barrier function, and vessel wall remodeling, by immunofluorescence microscopy, dye transfer and nanoparticle permeability experiments, and histology. To identify the role played by specific GCX components in EC function, some assays were performed on ECs with intact GCX and others were performed on ECs with experimentally degraded GCX. We also replaced degraded GCX components and assessed subsequent restoration of EC functions. Results demonstrated that the sialic acid (SA) component of cultured EC GCX in healthy flow is 2.66 mm thick and covers ~60% of the endothelial surface. SA thickness decreases in complex flow conditions by a significant 15%. In complex flow conditions, there is significant 58% drop in SA coverage. Heparan sulfate (HS) and hyaluronic acid GCX components were modulated differently in correlation to flow conditions. Vasoregulation, communication, barrier function, and blood vessel wall remodeling were all found to correlate to GCX composition. GCX repair by treating the cells with exogenous HS (and a co-factor) restored barrier function and recovered communication, suggesting that targeting the GCX may be a promising approach to reversing EC dysfunction and vascular disease progression.

S22-2 Loss of the Retinal Endothelial Glycocalyx in Diabetes

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The endothelial glycocalyx serves many purposes, one of which is a contribution to barrier function, with molecules greater than 40 kD in size having restricted access to the endothelial cell surface and junction. In the current experiments, we sought to investigate the effects of diabetes and hyaluronidase (HAase) on the thickness of the endothelial glycocalyx layer in the mouse retina. Two different fluorescent dextrans (4 kD-FITC & 155 kD-TRITC) were injected into control nondiabetic mice, diabetic Ins2Akita mice, and also into nondiabetic mice given an intravascular injection of HAase, which can degrade hyaluronic acid (HA) in the glycocalyx. Glycocalyx thickness was measured as one-half the difference in the lumen diameter filled by the 4 kD dye minus that of the 155 kD dye (the latter having limited transport into the glycocalyx). Additionally, a vascular leakage index was calculated from the tissue fluorescence intensity of each dye relative to the vascular intensity (tissue/vessel). We found that diabetes reduced the retinal endothelial glycocalyx layer significantly in the

arterioles ($p<0.01$), but not in the venules, with the same pattern also found in the experiments in which HAase was infused into control mice. Furthermore, wheat germ agglutinin staining of the vessel wall, in which the lectin nonspecifically binds HA residues, was found to be greater in arterioles than in venules, which could explain the greater effect of HAase on the arterioles. HAase increased vascular leakage of both sized dextrans into the surrounding tissue. In summary, our findings indicate that diabetes reduces the thickness of the retinal endothelial glycocalyx, containing HA, which may play a significant role in blood retinal barrier function.

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S22-3 Endothelial glycocalyx restoration by growth factors in diabetic kidney disease

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The endothelial glycocalyx (eGlx) constitutes the first barrier to protein in all blood vessels. This is particularly noteworthy in the renal glomerulus, the ultrafiltration barrier. Any leakage of protein, such as albumin, across glomerular capillaries results in albumin in the urine (albuminuria), a hall mark of kidney disease. We demonstrate that targeted damage to the glomerular eGlx, using enzymes, results in a direct increase in glomerular albumin permeability using an ex vivo isolated glomerulus assay. This was confirmed by a reduction in eGlx coverage and/or depth using quantitative electron microscopy. In rodent models of diabetes, we also demonstrated a loss of glomerular eGlx which was associated with increased albumin permeability. Treatment with paracrine growth factors such as vascular endothelial growth factor (VEGF) C and angiopoietin-1 could rescue albumin permeability and restore glomerular eGlx. In cultured glomerular endothelial cells, these growth factors promoted the synthesis of the eGlx component, hyaluronic acid, and upregulated its biosynthesis enzyme, hyaluronic acid synthase 2 (HAS2). Further, inhibition of HAS2 increased glomerular albumin permeability ex vivo. HAS2 appears to have potential as a therapeutic target in diabetic kidney disease and this will be the focus of future work.

S22-4 Modification of renal macrophage signalling via MCP-1 inhibition reduces albuminuria in diabetic nephropathy

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Recently it was shown that interception of MCP-1 with the Spiegelmer emapticap pegol (NOX-E36) resulted in reduced albuminuria in type 2 diabetic nephropathy patients. Pro-inflammatory macrophages express cathepsin L, which activates heparanase, thereby

degrading heparan sulfate (HS), one of the major endothelial glycocalyx components. Here we hypothesize that MCP-1 inhibition reduces albuminuria via influencing macrophage function, resulting in reduced heparanase activity and restoration of endothelial glycocalyx dimensions. ApoE KO mice were made diabetic and received a high cholesterol diet (0.15%) for 10 weeks, resulting in proteinuria and diabetic glomerular lesions. Mice were then treated for 4 weeks with mNOX-E36 or control. Cationic ferritin (CF) binding to the negatively charged HS was imaged using TEM and F4/80, cathepsin L and heparanase expression using IHC. Cytokine production upon LPS was measured in isolated kidney macrophages. mNOX-E36 treatment (4wk) reduced albuminuria and was accompanied with reduced glomerular cathepsin L and heparanase expression and increased CF. With equal numbers of glomerular macrophages their functionality was remarkable changed (decreased release of IL6 versus IL10), demonstrating an anti-inflammatory phenotype. In conclusion, MCP-1 inhibition by mNOX-E36 decreases albuminuria in diabetic nephropathy mice. The accompanied induction of anti-inflammatory macrophages resulted in reduced local heparanase presence and glycocalyx restoration.

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