Syndecan-1 Modulates the Endothelial Shear Mechanotransduction Response and Inflammatory Phenotype

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Statement of Purpose: Atherosclerosis is the principle cause of cardiovascular disease, which accounts for one out of every three deaths annually in the United States. It begins as a chronic inflammatory condition and thus early treatments to reduce chronic vascular inflammation could help prevent the onset and reoccurance of atherosclerosis. Shear stress has long been known to modulate endothelial phenotype, particularly in regards to inflammation; however, the initially sensory mechanisms that drive these changes in phenotype remain unclear. By analyzing the mechanobiological cues utilized by endothelial cells to maintain a healthy phenotype, we hope to investigate the potential for a novel class of therapeutics to reduce the occurance of atherosclerotic plaques. To this end, we have developed an in-vitro model consisting of wild type and endothelial cells that have been knocked out for the glycocalyx protein syndecan-1. Additionally, we have used a lentiviral vector to reestablish sdc-1 in the S1KO line under an upregulated promoter (pSyn1). Using this in-vitro model we examined the role of syndecan-1 in mechanotransduction pathways relating to the adhesion of circulating leukocytes.

Methods: *Cell Culture*- Primary microvascular endothelial cells were isolated from the lungs of wild type and syndecan-1 knockout mice by mechanical mincing and collagenase digestion. Cells were confirmed for endothelial purity by twice sorting with CD31-bound Dynabeads (Invitrogen) as well as uptake of fluorescently labeled Ac-LDL and Tie-2 mRNA expression. Using a lentiviral vector, we also reintroduced sdc-1 into the S1KO line with an upregulated promotor (pSyn1). *Real-time PCR*- Cells were grown to confluence and then exposed to either static conditions or flow at 12 dynes/cm^2 for 24 hours in six-channel plates (μ-Slide VI$^{0.4}$; Ibidi, LLC) using our custom-designed system[1]. They were then lysed and the mRNA isolated with a commercially available kit (Qiagen). Gene expression was measured via real-time PCR after mRNA conversion to cDNA and normalized to GAPDH as a control. *Leukocyte Adhesion Assay*- Confluent monolayers of WT, S1KO and pSyn-1 endothelial cells were grown in slide mounted flow chambers (Ibidi) and then stimulated with the addition of 10 ng/mL TNF-α for 4 hours. The flow chambers were positioned on a inverted microscope and CellTracker dye (Invitrogen) labeled THP-1 cells (5 x 10^5 cells/mL) were perfused through the chamber with a syringe pump (Kent Scientific) for 5 min at a controlled flow rate to generate a shear stress of 0.5 dynes/cm^2. The entire period of perfusion was recorded as a digital video and analyzed to determine the number of rolling THP-1 cells over monolayers in three 90-second intervals. After 5 min the flow was changed to MCDB-131 media to wash out unadhered cells and the plates were imaged in 8 separate locations. The number of cells adhered per field of view was by quantified using Metamorph software.

Results: Under static and atheroprotective flow conditions we found a 200-300 fold increase in ICAM-1 expression in sdc-1 knockout cells in comparison to WT cells (**Fig. 1A**). Under atheroprotective flow, sdc-1 knockout cells had over four-fold more VCAM-1 gene expression. In addition, while ICAM-1 levels remained elevated, VCAM-1 and E-selectin expression levels were reduced in the pSyn1 model. We next examined the functional consequences of sdc-1 knockout on monocyte adhesion in-vitro. About 50-fold more monocytes adhered to sdc-1 knockout endothelial monolayers than to WT monolayers under identical flow conditions (**Fig. 1B-1C**). More cells were also observed to roll on sdc-1 knockout monolayers during the flow adhesion assay (**Fig. 1D**). Overexpression of sdc-1 in the knockout cells using lentiviral transduction reduced the number of adhered monocytes by about half in comparison to sdc-1 knockout cells (**Fig. 1C-1D**).

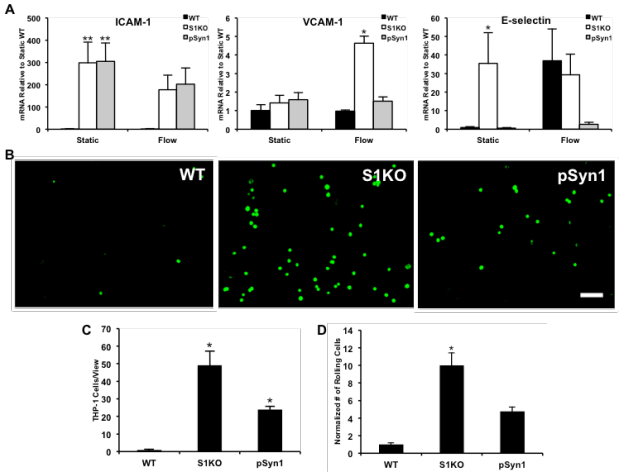

Figure 1. A) ICAM-1, VCAM-1, and E-selectin levels are increased in the S1KO model. B-D) More flowing monocytes adhere to the S1KO model, while restoration of sdc-1 shows a partial recovery.

Conclusions: These findings illustrate that syndecan-1 has a significant effect on the mechanobiology of the vascular endothelium and its absence results in a highly inflammatory phenotype. Interestingly, by reintroducing sdc-1 to the S1KO model we were able to partially recover the wild type phenotype. These results consisted of both analyzing the gene expression of ICAM-1, VCAM-1, and E-selectin as well as conducting our functional leukocyte adhesion study. These results may yield novel approach to drug-releasing material therapies to reduce the chronic inflammatory conditions found in early atherosclerotic plaques. Future studies would include release of syndecan-1 proteoliposomes in atherosclerosis-susceptible ApoE -/- mice to examine if these findings are translatable to an in-vivo model.

References: [1]Voyvodic PL, et al. Lab on a Chip. 2012;12(18):3322-3330.