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TRANSCRIPTOME PROFILING IN PORCINE ARTERIES TO IDENTIFY NOVEL SHEAR-RESPONSIVE REGULATORS OF ENDOTHELIAL CELL FATE

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doi:10.1136/heartjnl-2013-304019.170

Introduction Atherosclerosis develops predominantly at regions of the arterial tree that are exposed to disturbed blood flow, which generates low, oscillatory shear stress (WSS) at the lumen. EC at low shear, lesion-prone regions are characterized by an increased rate of apoptosis, thus providing a potential explanation for the distinct spatial localization of atherosclerosis. To understand the interaction between mechanosensitive and apoptotic signalling networks we used microarray technology coupled to computational fluid dynamics to identify genes differentially expressed at high or low WSS regions of the porcine aorta. We examined whether putative regulators of apoptosis can be activated by flow *in vitro* and studied their function using cultured EC.

Methods WSS was mapped in porcine aortae by accurately defining the geometry and flow characteristics using a 3 Tesla MRI (Siemens). The blood behavior was modeled using Fluent 6.2 (ANSYS). 3D shear stres maps were generated using an in-house code to inform isolation of EC from high and low shear regions for subsequent microarray analysis (Affymetrix). Differentially expressed genes were identified using Genespring software (Agilent) and annotated using the DAVID database to identify putative regulators of apoptosis. The influence of shear stress on the expression of putative regulators of apoptosis was studied using cultured porcine aortic EC (PAEC) exposed to flow using an orbital system. Gene function was studied in sheared EC using siRNA-based approaches.

Results Computed WSS maps revealed great spatial heterogeneity and challenged common assumptions about the mechanical conditions at susceptible and protected regions. Microarray analysis of ECs isolated from the aortic arches of 5 pigs identified 764 differentially expressed genes that influence diverse physiological activities. Functional annotation of these transcripts highlighted the presence of 41 molecules with an inferred role in the regulation of apoptosis. We validated this gene set by quantitative RT-PCR analysis which confirmed that 88% microarray 'hits' were differentially expressed.

Moreover, 87% differentially expressed genes were induced by WSS in cultured porcine aortic EC. We selected 2 candidate regulators of apoptosis for functional screening using *in vitro* systems: PERP and PDCD2L. Staining for active caspase-3 and TUNEL revealed that EC apoptosis was significantly enhanced in EC exposed to oscillatory WSS for 72 h compared to cells exposed to uniform flow. Silencing of PERP and PDCD2L reduced apoptosis in EC exposed to oscillatory WSS.

Conclusions We conclude that shear stress influences EC viability through transcriptional mechanisms that involve the novel apoptosis regulators PERP and PDCD2L. Further work is required to define the molecular mechanisms that underly the induction of apoptosis by these molecules.