

novel regulators of EC dysfunction, PERP which is a target of the pro-apoptotic transcription factor p53, and PDCD2L which has sequence similarity with the apoptosis effector RP8. Future work will be focused on defining the mechanism by which PERP and PDCD2L induce apoptosis in ECs.

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IDENTIFICATION OF NOVEL SHEAR STRESS-RESPONSIVE REGULATORS OF ENDOTHELIAL CELL DYSFUNCTION USING THE ZEBRAFISH MODEL

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Introduction Atherosclerosis, an underlying cause of cardiovascular disease, is a focal disease of arteries. Haemodynamic shear stress exerted on the endothelium by the flowing blood plays an important role in the pathogenesis of atherosclerosis and spatial distribution of atherosclerotic lesions. Low, oscillatory shear stress promotes atherosclerosis by regulating endothelial cell (EC) viability and function, while high shear is athero-protective. To elucidate the molecular mechanisms underlying the effects of shear stress on EC physiology, we used microarray technology coupled to computational fluid dynamics to define the EC transcriptome at low and high shear regions of the porcine aorta. The study identified 60 putative regulators of apoptosis that were differentially expressed at the two aortic regions. We hypothesised that this gene set includes key regulators of EC survival in response to haemodynamic forces.

Methods To assess the function of specific genes in ECs and their potential role in the response to haemodynamic force, we have used zebrafish, which is an emerging vertebrate model in vascular biology. We selected five putative regulators of EC apoptosis that were differentially expressed between high and low shear stress region of the porcine aorta. Their expression was transiently knocked down in zebrafish embryos with gene-specific morpholino antisense oligonucleotides, while blood flow was modulated using the *silent heart* model (cardiac troponin T deficiency) or tricaine treatment. The effect of gene silencing on EC apoptosis was examined by immunofluorescent staining using antibodies that recognise active caspase 3 and by TUNEL assay.

Results Our study reveals that cessation of blood flow promotes EC apoptosis in zebrafish embryos. Morpholino-mediated knockdown of two candidate genes from the microarray analysis, TP53 apoptosis effector (*PERP*) or programmed cell death protein 2-like (*PDCD2L*), reduced apoptosis in ECs exposed to flow cessation by approximately 30% ($p < 0.0001$ for both *PERP* or *PDCD2L*). These data indicate that *PERP* and *PDCD2L* have a role in promoting EC apoptosis in the absence of haemodynamic forces.

Conclusions We have established a platform for functional screening of flow-regulated genes in zebrafish. Initial screening identified two