miRNA-1237-3p modulates shear-stress dependent aortic valve endothelial inflammation

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Background

- Calcific Aortic Valve Disease (CADV) is a significant cause of mortality in aging population with only one current viable treatment: surgical valve replacement.
- CADV is defined by pathological changes in the valve which begin with aortic valve sclerosis that progress to aortic valve stenosis.
- Calcification preferentially occurs on the aortic side of the valve (fibrosa), where the endothelial cells are subjected to disturbed blood flow (OS), and exhibit an inflammatory phenotype.
- The ventricular side experiences laminar shear stress (LS) and exhibits an anti-inflammatory phenotype and it is rarely found to be calcified.
- miRNAs are small nucleotide sequences that are part of the 3’ untranslated region (UTR) of mRNA leading to the degradation of that mRNA or inhibition of protein translation.
- We seek to determine miRNAs and genes that are differentially expressed in the valve in response to shear stress and their potential as therapeutic targets to prevent aortic valve calcification.

Array and validation

- miR-1237-3p is increased in laminar unidirectional flow in HAECs and in the fibroin of porcine aortic valves.
- Expression of miR-1237-3p was assessed by qPCR that above that in control conditions that with laminar shear.
- The aorta was harvested from healthy pigs and were sheared with a flow rate of 20 dynes/cm² and OS was induced at a 5 dynes/cm². After 24 hours, cell alignment as shown in the first image was conserved in laminar shear stress (LS).
- Endothelial-enriched RNA is collected from porcine valves by using an isopropanol-soaked nitrocellulose membrane over the endothelial side of the valve.
- miRNA overexpression and inhibition was achieved using pre-miRNAs and LNA-anti-miRs from Life Technologies and Exiqon respectively at a concentration of 5 pmol/μl according to observed efficacy.
- Monocyte binding assays were conducted by incubating HAECs with the human monocytic line. 10 images from each well were taken and the average was used as the value for that sample.

Materials and Methods

- Laminar Shear Stress (LS)
- Oscillatory shear stress (OS)

Results

- In vitro shear stress experiments were carried out using cone-and-plate shear system. LS was induced by parallel force of 20 dynes/cm², and OS was induced at a 5 dynes/cm². After 24 hours, cell alignment as shown in the first image was conserved in laminar shear stress (LS).
- Endothelial-enriched RNA is collected from porcine valves by using an isopropanol-soaked nitrocellulose membrane over the endothelial side of the valve.
- miRNA overexpression and inhibition was achieved using pre-miRNAs and LNA-anti-miRs from Life Technologies and Exiqon respectively at a concentration of 5 pmol/μl according to observed efficacy.
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miRNA-1237-3p regulates critical targets of endothelial dysfunction such as CxCL2, CxCL12, NOX4 or THBS1

miR-1237-3p is an anti-inflammatory miRNA that regulates inflammation in HAECs as well as specific to porcine aortic valves.
- miR-1237-3p is a novel shear-sensitive miRNA in HAECs as well as specific to porcine aortic valves.

Summary & Working Hypothesis

- We have found CxCL2, CxCL12, NOX4 and THBS1 to be shear-sensitive in HAECs as well as targets of miR-1237-3p. These genes are key regulators of endothelial dysfunction and may provide insight in the role of miR-1237-3p in valvular endothelial dysfunction.

miR-1237-3p may be a novel therapeutic target for CAVD by inhibiting shear-dependent endothelial inflammation.

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