Working Title

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Key Words: Atherosclerosis • Wnt Signalling Pathway • βeta-catenin • Shear Stress • Human Umbilical Vein Endothelial Cells (HUVECs) • Angiopoietin-2 • Thrombospondin-1

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Introduction

Flow

Flow During Development

Developmental Proteins / mechanosensors

Endothelial

Atherosclerosis

WSS

Pathway

Does Axin, Angp2, Thrombosin-2 change if Wnt is inhibited?

Hypothesis

XAV-939 Wnt/Beta Catenin inhibitor, acts by inhibiting tankyrase

Methods

Orbital Shaker

HUVECs were cultured in M199 complete growth medium until ~80% confluent. Cells were then washed with warmed PBS and incubated with 1ml of trypsin until cells thoroughly detached. Cells were transferred to a falcon tube with M199 and spun for 5 minutes at 400g. The supernatant was discarded, and cells were re-suspended in M199 media and transferred to 10mm radius 6 well plates. Once confluent, 3ml of 1% DMSO in M199 was added to one half of the plates, and 3ml of 1% XAV939 Wnt inhibitor in M199 to the other half. Cells were subjected to flow using a orbital shaker at 210 rpm for 72 hours, with the exception of a static controls.

mRNA Isolation and qPCR

Media was removed from the plates and cells were washed with cold PBS. Cells were isolated from the periphery and centre of the plates with PBS and centrifuged for 5 minutes at 400g to remove the supernatant. Total mRNA was extracted using the

RNEasy Mini Kit (Qiagen). The amount of isolated mRNA was determined spectrophotometrically. cDNA synthesis was performed using the Verso cDNA Synthesis Kit (Thermo Scientific) with 5.5µl of 0.64% of mRNA. ANGPT2, AXIN2, THSB1, and HPRT1 mRNA was quantified using StepOne qPCR (Thermo Scientific) using oligonucleotide qPCR primers from Ensembl (Howe et al., 2020) (Table 1).

Table 1. Oligonucleotide qPCR primers from Ensembl.

Gene	Direction	Sequence
ANGPT2	L	CGGCTGTGATGATAGAAATAGGGA
	R	GTTCCAAGAGCTGAAGTTCAAGTC
AXIN1	L	TGTCACTTACTTTTTCTGTGGGGA
	R	TGTCACTTACTTTTTCTGTGGGGA
HPRT1	L	TTGGTCAGGCAGTATAATCC
	R	GGGCATATCCTACAACAAC
THSB1	L	AAAGATGGAGAATGCTGAGTTGGA
	R	GGTTCCAAAGACAAACCTCACATT

Results

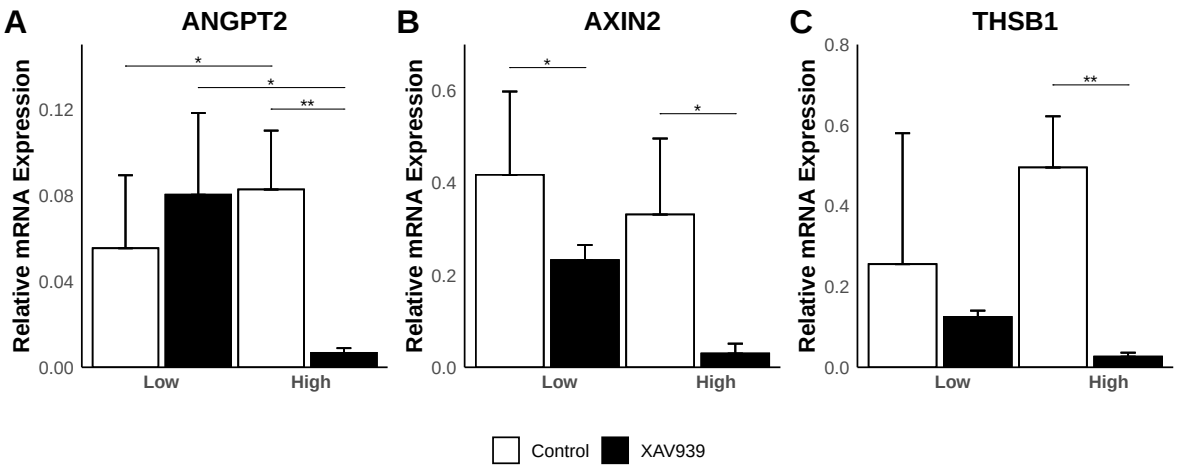

Figure 1. Relative mRNA expression of angiopoeitin-2, axin-2, and thrombospondin-1 from HUVECs exposed to high and low oscillatory shear stress (OSS). Normalised to the HPRT control and relative to the static control.

Discussion

Atheroprotective gene expression

Limitations of orbital shaker = imporve method

Future

epigenetics

look at proliferation, apoptosis, senescence, inflammation = PERP, p53

look at vascular repair = wound scratch assay?

look at emt = slug/snail?

Acknowledgements

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References

Howe, K. L. et al. (2020). Ensembl 2021. *Nucleic Acids Research*, 49 (D1), pp.D884–D891. [Online]. Available at: doi:10.1093/nar/gkaa942.