Working Title

Natasha Hopkins

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Univeristy of York, UK

Project Director

Dr. Richard Maguire

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

(250 Words)

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 flasks until ~80% confluent. Cells were then washed with warmed PBS and incubated with 1ml of trypsin until cells thoroughly detached. M199 media was added to the cells, before being transferred to a falcon tube 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% 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 control.

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 using 350µl of PBS, before centrifuging for 5 minutes at 400g and removing the supernatant. Total mRNA was isolated using the RNEasy Mini Kit (Qiagen) and concentration was determined using a

spectrophotometer. mRNA was reverse transcribed to cDNA using the Verso cDNA Synthesis Kit (Thermo Scientific). The oligonucleotide qPCR primers were obtained from Ensembl (Howe et al., 2020) (Table 1).

Table 1. Oligonucleotide qPCR primers from Ensembl.

Gene	Direction	Sequence
ANGPT2	L	CGGCTGTGATGATAGAAATAGGGA
	R	GTTCCAAGAGCTGAAGTTCAAGTC
AXIN	L	TGTCACTTACTTTTTCTGTGGGGA
	R	TGTCACTTACTTTTTCTGTGGGGA
HPRT1	L	NA
	R	NA
THSB1	L	AAAGATGGAGAATGCTGAGTTGGA
	R	GGTTCCAAAGACAAACCTCACATT

Results

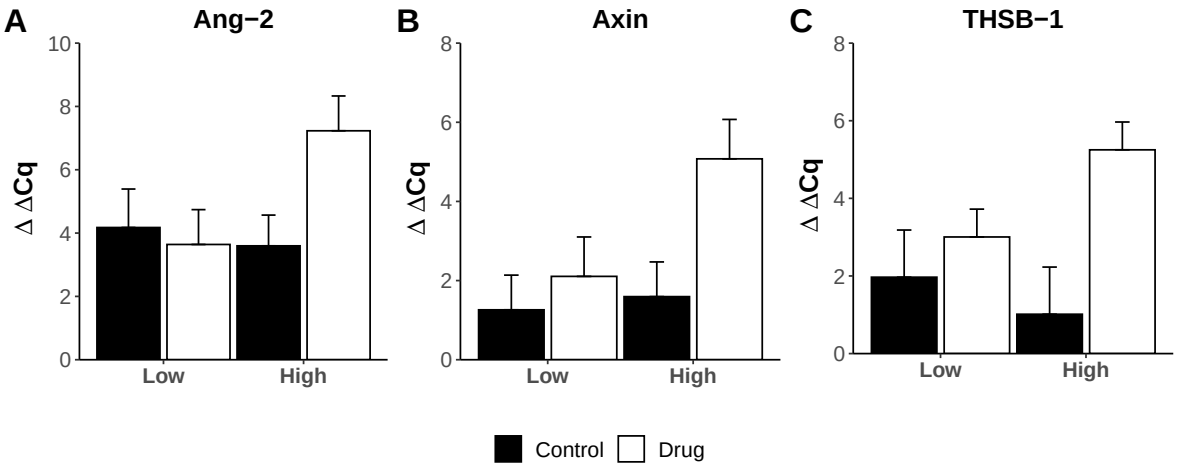

Figure 1. Relative to HPRT control.

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.