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

(250 Words)

Introduction

Atherosclerosis

Flow

Flow During Development

Developmental Proteins / mechanosensors

Endothelial

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 complete growth medium containing M199, sodium bicarbonate, pen-strep, amphotericin B, Hi-FBS, endothelial cell growth supplement (ECGS), and herparin. When ~80% confluent, cells were incubated with 1ml of trypsin until cells thoroughly detached, and neutralised with 9ml of M199. Cells were spun for 5 minutes at 400g to discard the supernatent, then re-suspended in M199 media before tranfering to 10mm radius 6 well plates. Once confluent, 3ml (Warboys et al., 2019) of 0.1% DMSO in M199 or 0.1% XAV939 in M199 were each added to half of the plates (Zhu et al., 2017). Cells were then subjected to flow using a orbital shaker at 210 rpm for 72 hours, with the exception of a static controls.

mRNA Isolation and qPCR

Cells were isolated from the periphery and centre of the plates with cold PBS and centrifuged for 5 minutes at 400g to remove the supernatant. Total mRNA was extracted using the RNEasy Mini Kit (Qiagen) and the concentration was determined spectrophotometrically. cDNA synthesis was performed using the Verso cDNA Synthesis Kit (Thermo Scientific) with 5.5µl of 0.01067% mRNA. *ANGPT2*, *AXIN2*, *THSB1*, and *HPRT1* mRNA was quantified using StepOne qPCR (Thermo Scientific) with SYBR

Green, 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

Statistical Analysis

Relative expression is expressed as $2^{\Delta\Delta Ct}$ fold change ± SEM. Normality was determined with Kolmogorov-Smirnov Tests. Multiple-comparison analysis was performed using Kruskal-Wallis Test followed by post-hoc Dunn's Test. Comparisons to the static control were performed using one-way ANOVA followed by post-hoc Tukey's HSD test. Statistical analyses were performed in R (R Core Team, 2018).

Results

HUVECs were treated with XAV939 and exposed to low (centre) and high (periphery) shear stress for 72 hours using an orbital shaker. Expression of *ANGPT2*, *AXIN2*, and *THSB1* were then quantified with qPCR.

For each gene, expression was significantly lower than the static control in all conditions. ($P < 0.0001$).

ANGPT2 expression is higher in cells exposed to high flow compared with low flow ($P < 0.05$). XAV393 upregulated *ANGPT2* in cells exposed to low stress ($P < 0.05$ versus control), however, downregulated *ANGPT2* in cells exposed to high stress ($P < 0.05$ versus control) (Fig. 1A).

Whereas in *AXIN2* and *THSB1*, expression did not significantly differ between the control cells exposed to low or high stress, and XAV393 downregulated cells exposed to both low and high stress ($P < 0.05$ versus control) (Fig. 1BC).

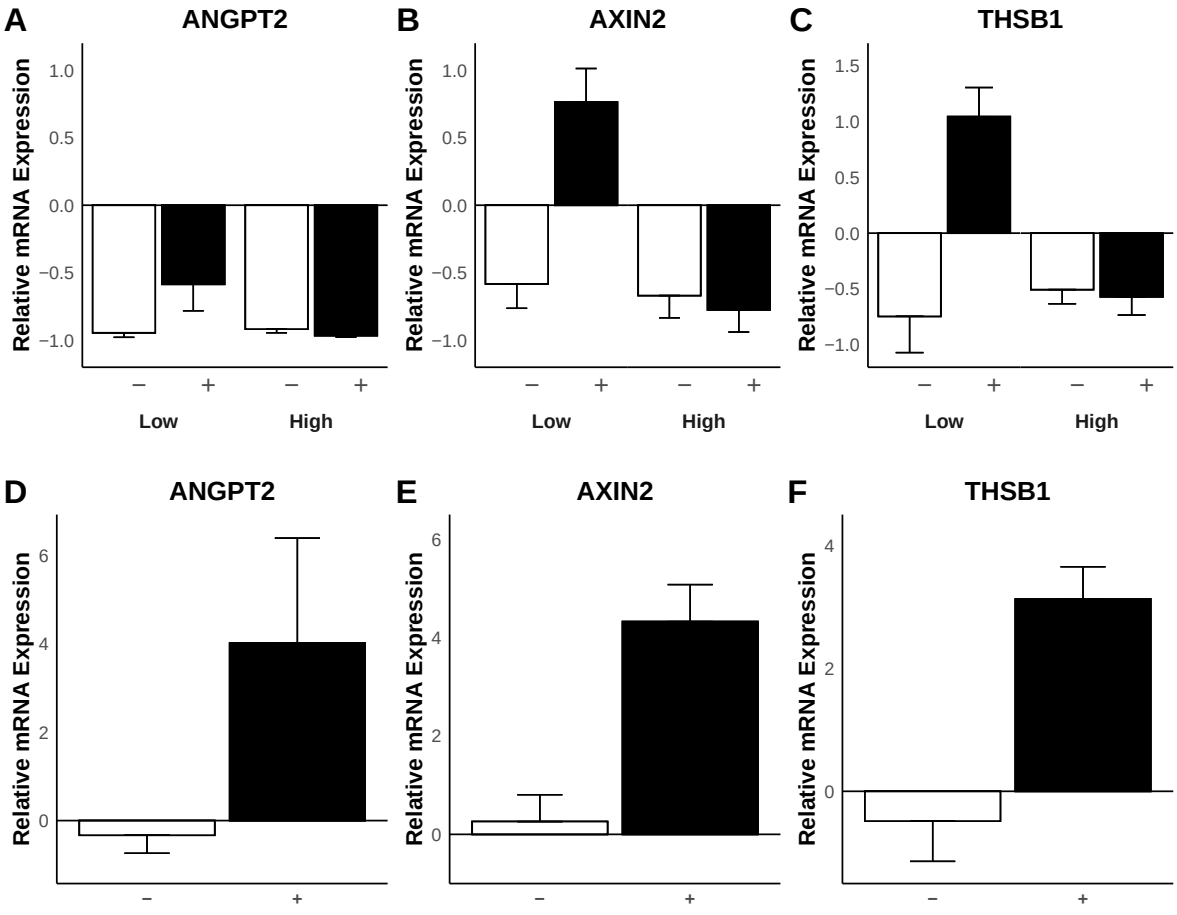

Figure 1. (**ABC**) Cells were treated with DMSO(-) or XAV939(+) and exposured to low or high shear stress. Levels of angiopoeitin-2 , axin-2, and thrombospondin-1 mRNA quantified by qPCR. Data is shown as fold change ± SEM, normalised to the HPRT control and relative to the static control. $^{**}P < 0.01$, and $^{*}P < 0.05$. (**DEF**) Under the same conditions, gene expression of cells exposed to low stress are compared to cells exposed to high stress. Data is shown as fold change ± SEM, normalised to the HPRT control and relative to the periphery control. $^{**}P < 0.01$, and $^{*}P < 0.05$.

Discussion

Atheroprotective gene expression

Limitations of orbital shaker = imporve method

Future

primer specificity test

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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