Working Title

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Key Words: Atherosclerosis • Wnt/βeta-catenin Signalling Pathway • Shear Stress • Orbital Shaker • Angiopoietin-2 • Thrombospondin-1

(250 Words)

Introduction

Atherosclerosis is an chronic inflammatory disease characterised by the formation of arterial plaques. Haemodynamic shear stress has been identified as a modulator of site specificity in atherosclerosis, which occurs preferentially in regions exposed to low, oscillatory shear stress. Whereas areas of high, laminar shear stress is atheroprotective (Timmins et al., 2017). Shear stress is an important factor in regulating gene expression in vascular endothelial cells (Ni et al., 2010), which is though to contribute to the susceptibility of plaque formation in atheroprone sites.

Multiple omics studies have implicated variations in flow with the regulation of developmental signalling pathways in atherosclerosis, including the Wnt Pathway (Souilhol et al., 2019; Gelfand et al., 2011). Wnt is an evolutionarily conserved pathway, with a crucial role in axis patterning during embryonic development.

Wnt Pathway

Multiple studies have demonstrated that the Wnt signalling pathway is mechanosensitive (Cha et al., 2016; Franco et al., 2016).

ANGPT2 & THBS1

Angiopoietin-2 (ANGPT2) is a well established growth factor involved in angiogenesis.

Thrombospondin-1 (THBS1) is an anti-angiogeneic.

- What are they?
- Previous studies

Hypothesis

A study is yet to distinguish expression of ANGPT2 and THBS1 in low and high shear stress in human umbilical vein endothelial cells (HUVECs), and whether these genes are mediated by Wnt signalling. We addressed this using an orbital shaker model, along with canonical Wnt inhibitor, XAV939.

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, Ghim and Weinberg, 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 normalised to the HPRT control. Normality was determined with Kolmogorov-Smirnov Tests. Comparison analysis was performed using the Student's *t*-test. All analyses were performed in R (R Core Team, 2018).

Results

In the orbital shaker system, low shear stress downregulated the expression of AXIN2, ANGPT2, and THBS1 in human umbilical vein endothelial cells. Expression of *AXIN2*, a known Wnt target, decreased by 0.28-fold in low shear stress. Similarly, ANGPT2 was decreased by ~0.15-fold, and THBS1 was decreased 0.12-fold.

Exposure to LSS with the addition of a Wnt inhibitor (XAV939) intensified the expression of *AXIN2* by 18.8-fold, ANGPT by 33.4-fold, and THBS1 by 35.6-fold. Conversely, XAV929 decreased expression in high shear stress. AXIN2 decreased 0.68-fold, ANGPT2 by 0.42-fold, and THBS1 by 0.87-fold.

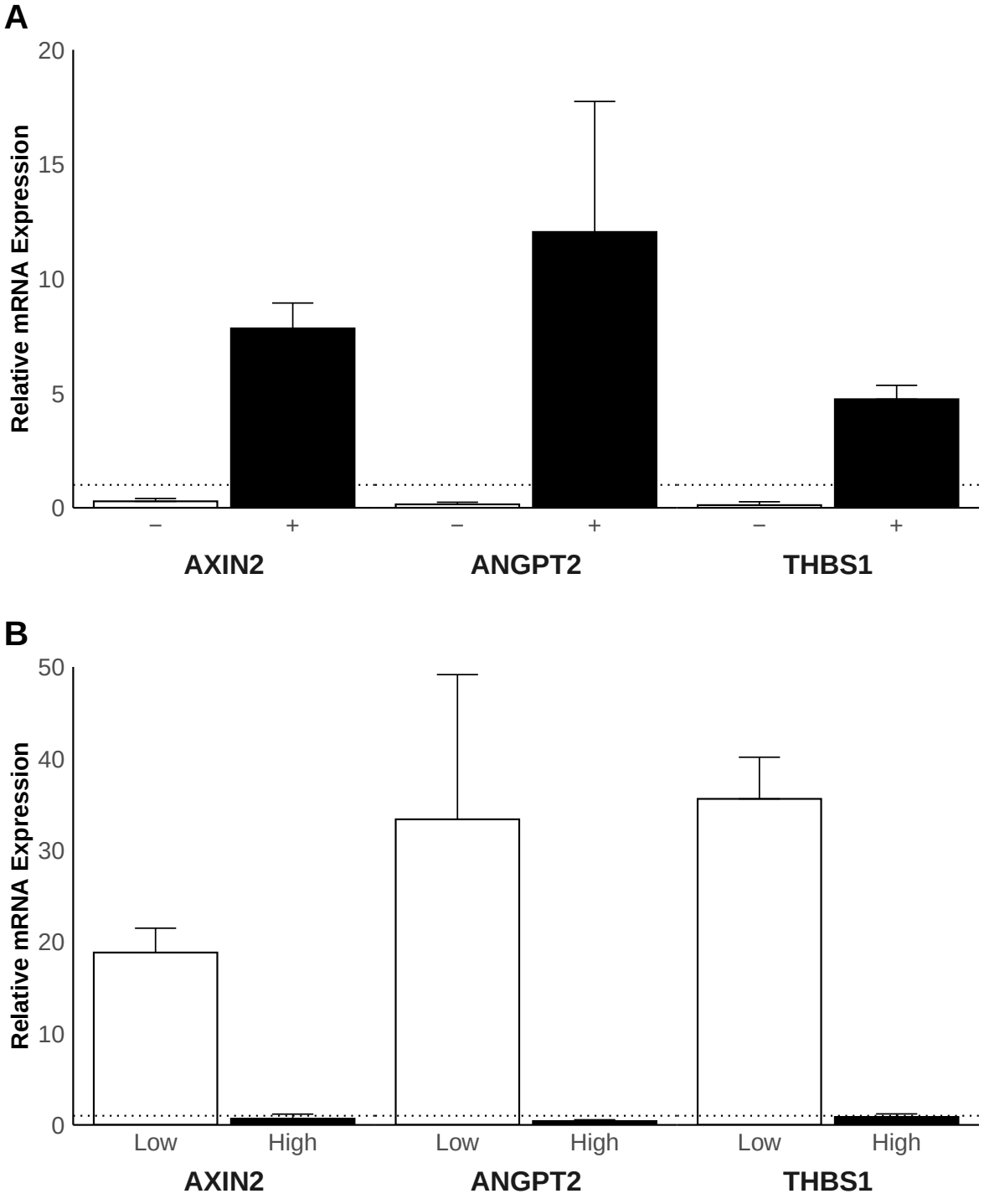

Figure 1. Cells were treated with DMSO(-) or XAV939(+) and exposed to low or high shear stress. Levels of angiopoeitin-2 , axin-2, and thrombospondin-1 mRNA quantified by qPCR. (**A**) Data is shown as fold change ± SEM of low shear stress relative to high shear stress. (**B**) Data is shown as fold change ± SEM of XAV939 relative to DMSO.

Discussion

Summarise Method & Results

Inhibitors & Axin

Unreliable Data

Refine Methods

- Methods used in other studies

Future Plans

Acknowledgements

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References

1. Cha, B. et al. (2016). Mechanotransduction activates canonical Wnt/?-catenin signaling to promote lymphatic vascular patterning and the development of lymphatic and lymphovenous valves. *Genes & Development*, 30 (12), pp.1454–1469. [Online]. Available at: doi:10.1101/gad.282400.116.

2. Franco, C. A. et al. (2016). Non-canonical Wnt signalling modulates the endothelial shear stress flow sensor in vascular remodelling. *eLife*, 5. [Online]. Available at: doi:10.7554/elife.07727.

3. Gelfand, B. D. et al. (2011). Hemodynamic Activation of ?-Catenin and T-Cell-Specific Transcription Factor Signaling in Vascular Endothelium Regulates Fibronectin Expression. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 31 (7), pp.1625–1633. [Online]. Available at: doi:10.1161/atvbaha.111.227827.

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

5. Ni, C.-W. et al. (2010). Discovery of novel mechanosensitive genes in vivo using mouse carotid artery endothelium exposed to disturbed flow. *Blood*, 116 (15), pp.e66–e73. [Online]. Available at: doi:10.1182/blood-2010-04-278192.

6. R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. [Online]. Available at: https://www.R-project.org/.

7. Souilhol, C. et al. (2019). Endothelial responses to shear stress in atherosclerosis: a novel role for developmental genes. *Nature Reviews Cardiology*, 17 (1), pp.52–63. [Online]. Available at: doi:10.1038/s41569-019-0239-5.

8. Timmins, L. H. et al. (2017). Oscillatory wall shear stress is a dominant flow characteristic affecting lesion progression patterns and plaque vulnerability in patients with coronary artery disease. *Journal of The Royal Society Interface*, 14 (127), p.20160972. [Online]. Available at: doi:10.1098/rsif.2016.0972.

9. Warboys, C. M., Ghim, M. and Weinberg, P. D. (2019). Understanding mechanobiology in cultured endothelium: A review of the orbital shaker method. *Atherosclerosis*, 285, pp.170–177. [Online]. Available at: doi:10.1016/j.atherosclerosis.2019.04.210.

10. Zhu, J. et al. (2017). Regulation of angiogenic behaviors by oxytocin receptor through Gli1-indcued transcription of HIF-1? in human umbilical vein endothelial cells. *Biomedicine & Pharmacotherapy*, 90, pp.928–934. [Online]. Available at: doi:10.1016/j.biopha.2017.04.021.