# miR-146a; a potential link connecting shear stress-induced activation of the pro-inflammatory and pro-proliferative Osteopontin & TLR-4 pathways.

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## Introduction

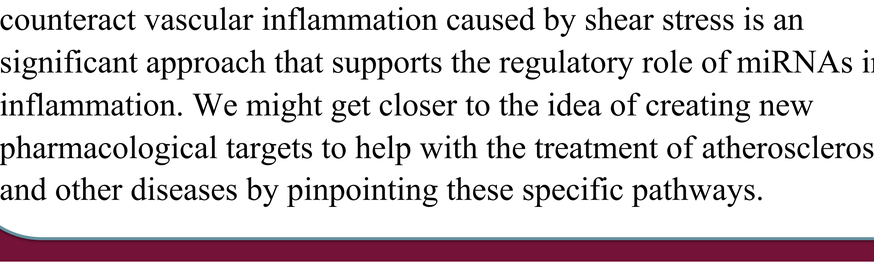
- This study investigates the miRNA system, an endogenous method of gene expression regulation that has been linked to vascular inflammation and Atherosclerosis.
- Shear stress (SS) is the force exerted by blood flow against the endothelial lining of blood vessels. Disturbed SS patterns lead to inflammation of endothelial cells. This inflammatory response contributes to the development of atherosclerosis. In contrast to areas of high unidirectional shear stress (USS), atherosclerotic lesions typically locate in areas of low oscillatory shear stress (OSS).
- A class of endogenous tiny RNA molecules known as microRNAs (miRNAs) has been linked to a variety of biological processes. Mature miRNAs help in the regulation of endogenous genes, primarily by translational repression.
- Transfection of miRNA mimics is a technique used to identify the targets and roles of particular miRNAs.
- Research in this area is ongoing, with studies focused on understanding the precise mechanisms of miR-146a regulation in response to shear stress, developing effective delivery methods, and evaluating the therapeutic potential of miR-146a modulation in animal models and clinical trials.

## Hypothesis

We would anticipate that the addition of Mir-RNA will reduce the expression of the proteins target proteins that are involved n the inflammatory pathways.

## Methodology

Human Aortic Endothelial Cells(HAECs) were subjected to simultaneous OSS and unidirectional shear stress(USS) control conditions using the standard orbital shaking model in vitro. HAECs subjected to acute OSS conditions isolated from the edge of the cell culture dish were compared to those isolated from the center of the cell culture dish that were subjected to USS conditions. miR-146a and scramble control were over-expressed using standard transfection protocols.






Human Aortic Endothelial Cells in vitro, treated with Mir146a. Dish treated through "shaking" in serum free media

## Impact & Significance

The goal is to investigate the processes involved in vascular inflammation by observing several target proteins that were impacted by these bands in Western Blots. Using miR-146a as a therapy to counteract vascular inflammation caused by shear stress is an significant approach that supports the regulatory role of miRNAs in inflammation. We might get closer to the idea of creating new pharmacological targets to help with the treatment of atherosclerosis and other diseases by pinpointing these specific pathways.

## Results

### Optimization of the Orbital-Shear-Model in Vitro

Fig. 1

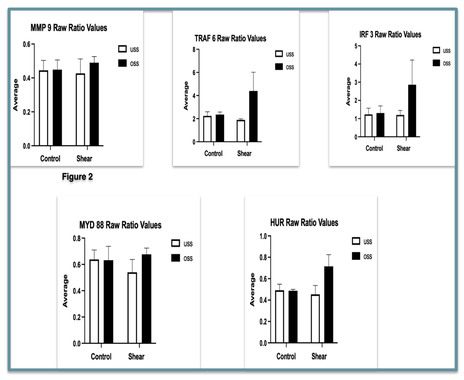



Figure 2

**Figure 1**: A series of data analysis of the Western Blotting include antibody MMP9, TRAF6, IRF 3, MYD88, HUR. All figures express that control set has no differences between OSS (center of the plate) versus USS (periphery of the plate). On the other hands, all figures show on the sheard plates, they have higher value of OSS and lower value of USS compared to the controls.

Fig. 2

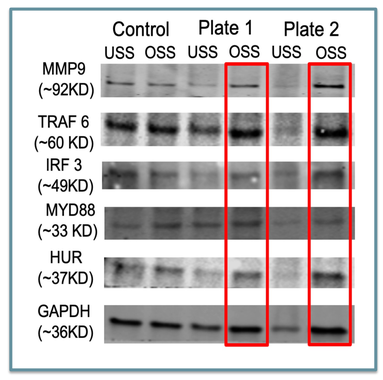



**Figure 2**: A series of Western Blotting analyses of cultured Human Aortic Endothelial cells (HAECs) shows the standard orbital shaker platform for induction of OSS (center of the plate) versus USS (periphery of the plate) flow condition. This shows expression on MMP9, TRAF6, IRF3, MYD88, and HUR.

Fig. 3

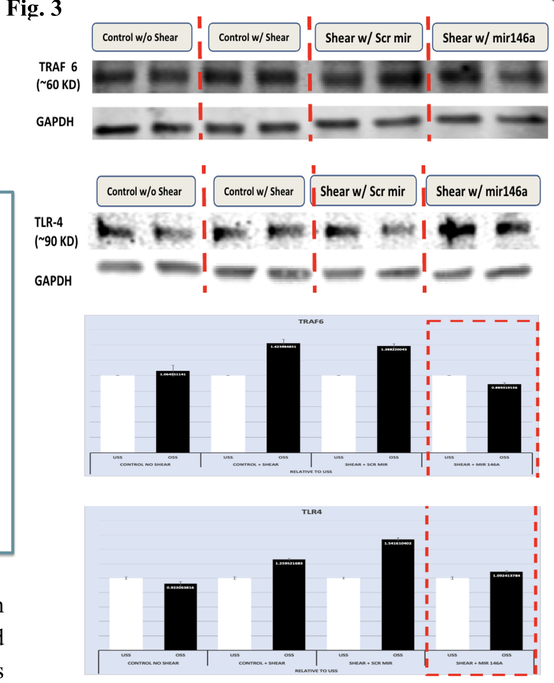



**Fig. 3**: Trend is consistent with TRAF-6; Our hypothesis predicts OSS results in increased expression of TRAF6 levels compared with USS, which was suppressed by miR-146a Overexpression compared with Scramble miR control. TRAF6 is a known direct target for Mir146a. TLR-4, which s the upstream receptor for TRAF6 shows a consistent similar trend as well.

Fig. 4

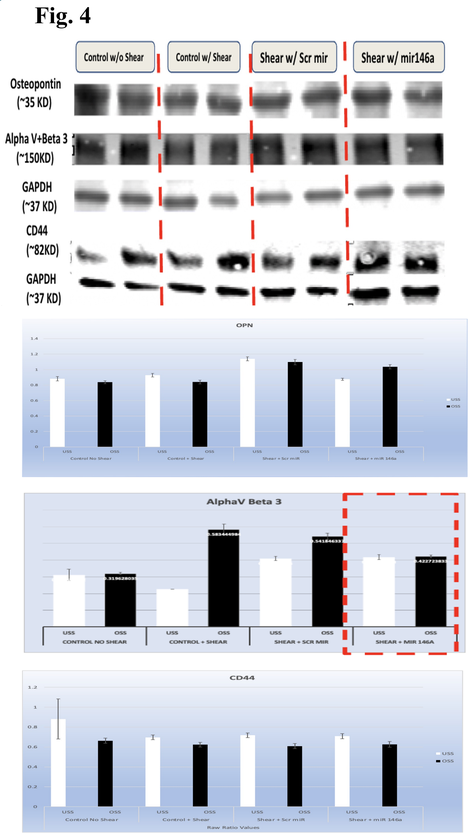



**Fig. 4:** Mir146a might suppress OPN, however, no consistent trend, compared to what we did see in the past. Further investigation is needed to confirm. A potential trend might be present with one of its receptors Alpha V Beta 3, but not with CD44.

Fig. 5

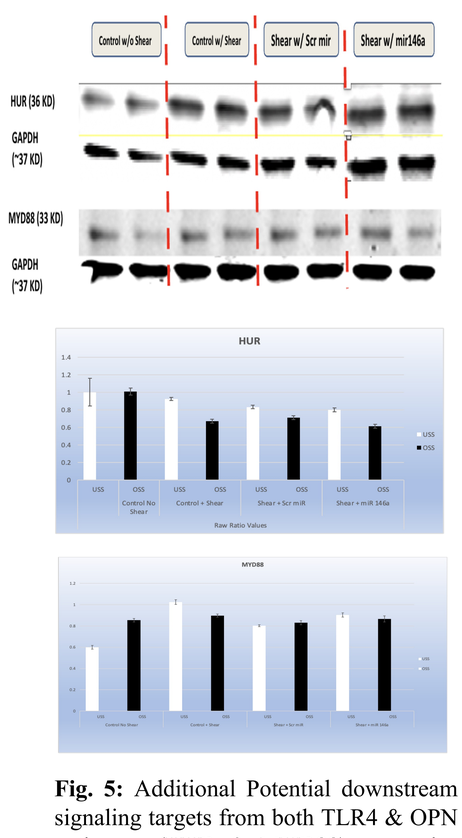



**Fig. 5:** Additional Potential downstream signaling targets from both TLR4 & OPN pathways (HUR & MYD88) were also screened if they can be targeted by miR146a in response to shear stress, but no consistent trend was observed.

Fig.6

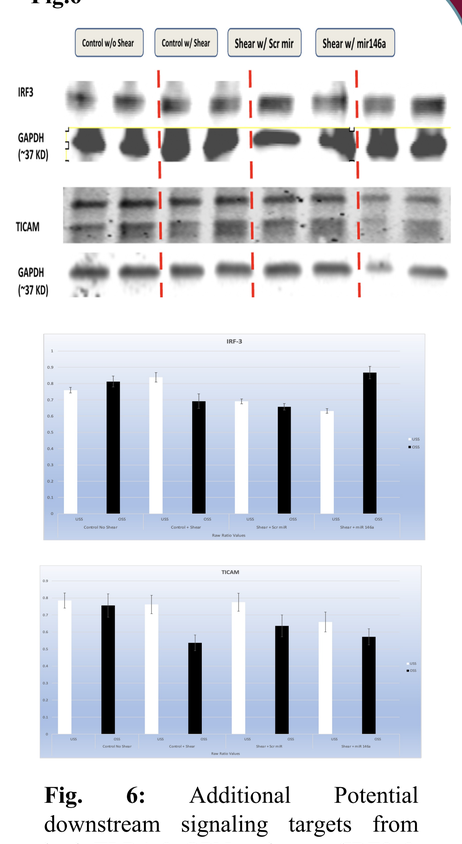



**Fig. 6:** Additional Potential downstream signaling targets from both TLR4 & OPN pathways (IRF3 & TICAM) were also screened if they can be targeted by miR146a in response to shear stress, but no consistent trend was observed.

## Conclusions

- Our findings suggest a potential role of miR-146a in suppressing shear-stress-induced endothelial inflammation.
- As of now, the most consistent positive trends we see that aligns with our hypothesis is for both TRAF-6 & its upstream receptor TLR4, which some of the known direct targets for Mir146a.
- Our results show a trend of over-expression of miR-146a blunted the OSS-induced expression of TLR4 & TRAF6 compared to scramble control.
- Further studies are needed to confirm the anti-atherogenic effects of miR-146a and its direct interaction with the OPN pathway and its receptor Alpha V Beta 3, and other targets as a foundation for developing innovative miR-based therapeutic modalities for atherosclerosis

## Acknowledgements and References

References:

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