



# SHP-2 Phosphatase Regulates ERK Phosphorylation Induced by Shear Stress



Joy E. Lin, Shu Chien, Jason H. Haga

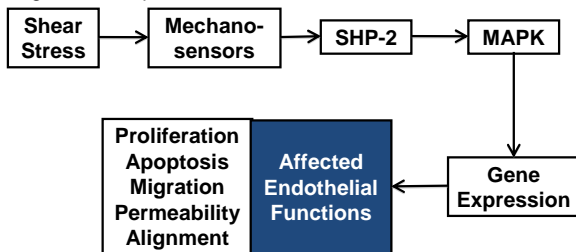
Department of Bioengineering, University of California, San Diego, La Jolla, CA 92092

## ABSTRACT

Vascular endothelial cells in the human body are continuously exposed to laminar shear stress forces caused by blood flow and pressure. These forces play a crucial role in health. For instance, atherosclerotic lesions occur preferentially at branch points of the arterial tree, where blood flow is disturbed. Thus, elucidating the molecular mechanism of pathways activated by shear stress is of great interest. Phosphatases, such as SHP-2, play a key role in such pathways. Previous research has shown that SHP-2 activates ERK phosphorylation in mouse fibroblasts. The objective of this study was to determine whether this relationship between the two molecules held under laminar shear stress in endothelial cells. Tests showed that ERK phosphorylation increased at ten minutes of shear and returned to control levels by sixty minutes. When SHP-2 was rendered inactive, however, ERK phosphorylation remained constant. This suggests that SHP-2 phosphatase regulates ERK-2 phosphorylation induced by laminar shear stress.

## INTRODUCTION

Previous research has shown that SHP-2, a protein-tyrosine phosphatase that is crucial to growth factor and integrin signaling, plays a key role in shear-induced molecular signaling. As shown in the diagram below, mechano-sensors of shear stress are known to affect SHP-2 phosphatase, which affects mitogen-activated protein kinase signaling, which in turn affects gene expression and endothelial cell functions. ERK-2 is a mitogen-activated protein kinase that regulates cell proliferation.



## OBJECTIVE

To elucidate the role of SHP-2 in ERK signaling in vascular endothelial cells under laminar shear stress.

## METHODS

- 1. Cell Culture:** Bovine aortic endothelial cells (BAEC) were cultured in growth media and maintained at 37°C with 5% CO<sub>2</sub>. Cells were seeded onto glass slides in four samples (IgG, C, 10', 60').
- 2. Transfection:** A mutant form of SHP-2 (SHP-2CS) and HA tagged ERK-2 were transfected via plasmids into one set of samples. A second set was transfected with empty vector as a negative control.
- 3. Laminar shear:** Cells were sheared for 10' and 60' or kept as static controls. The magnitude of shear used was 12 dyn/cm<sup>2</sup>, which is representative of the force exerted by blood flow in the aorta.
- 4. Protein assay:** The protein concentration of the samples was determined by the Bradford method. Equal amounts of protein (20µg) were then electrophoresed.
- 5. Immunoprecipitation:** HA-tagged ERK-2 was isolated from the transfected samples by incubation with an anti-HA antibody and captured with Protein A/G beads.
- 6. SDS-PAGE and transfer:** Proteins were separated based on size using polyacrylamide gel electrophoresis and subsequently transferred onto nitrocellulose.
- 7. Immunoblotting:** Nitrocellulose blots were blocked with 5% BSA in TBSt and then probed with antibodies specific for phospho-ERK and total ERK.

## RESULTS

As shown in Fig. 1, laminar shear stress induced a transient phosphorylation of ERK in endothelial cells. The phosphorylation increased at 10' and returned to static control levels by 60'.

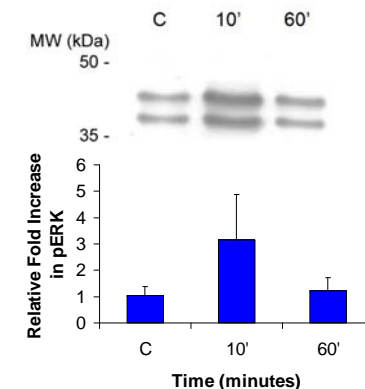


Fig. 1: The 10' and 60' samples were subject to laminar shear for 10 and 60 minutes respectively, while the control (C) sample was not subjected to shear stress. Results showed that at 10 minutes of shear stress, ERK phosphorylation increased more than three-fold before returning to control levels at 60 minutes.

## RESULTS (cont.)

Transfecting cells with SHP-2CS inhibited the shear-induced phosphorylation of ERK, as shown in Fig. 2.

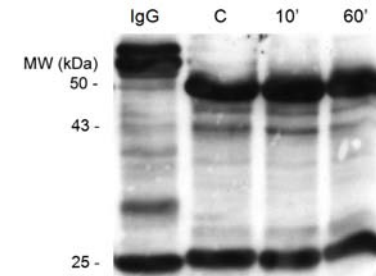


Fig. 2: Samples depicted are IgG, which served as negative control, C, which served as static control, 10', and 60', which represented samples that had been sheared for 10 and 60 minutes respectively. In contrast to Fig. 1, results showed no change in ERK phosphorylation.

## CONCLUSIONS

In the absence of interfering with SHP-2 signaling, endothelial cells responded normally, as demonstrated by the increase in ERK phosphorylation at 10'. In contrast, when SHP-2 signaling is disrupted, shear-induced ERK phosphorylation is suppressed. Thus, it can be concluded that SHP-2 signaling is critical to ERK-2 phosphorylation under laminar shear stress.

## REFERENCES

- 1.Li, Y. S., Haga, J. H., Chien, S. 2005. Molecular basis of the effects of shear stress on vascular endothelial cells, *Journal of Biomechanics*, Vol 38, 1949-1971.
- 2.Zhang, S. Q., Yang, W., Kontaridis, M., Bivona, T., Wen, G., Araki, T., Luo, J., Thompson, J., Schraven, B., Philips, M., Neel, B. 2004. Shp2 Regulates Src Family Kinase Activity and Ras/Erk Activation by Controlling Csk Recruitment, *Molecular Cell*, Vol 13, 341-355.
- 3.Chan, G., Kalaizidis, D., Neel, B. 2008. The tyrosine phosphatase Shp2 in cancer, *Cancer Metastasis Review*, Vol 27, 179-192.

## ACKNOWLEDGMENTS

Vascular Molecular Bioengineering Laboratory, Calit2