Confocal Microscopic Quantification of Vinculin Phosphorylation at Serine 722 in Vascular Endothelial Cells by Using Fluorescence Resonance Energy Transfer

Yu-Tsung Shih, Mei-Cun Wang, Pei-Li Lee, Jeng-Jiann Chiu.
Institute of Cellular and System Medicine
National Health Research Institutes
35 Keyan Road, Zhunan, Miaoli County, Taiwan
E-mail: ytshih@nhri.org.tw; jjchiu@nhri.org.tw

KEY WORDS: FRET, Molecular imaging, Endothelial cell, Vinculin phosphorylation, Flow

In the arterial system, regions of disturbed flow with oscillatory shear stress (OSS) are susceptible to atherosclerosis, whereas regions of laminar flow with pulsatile shear stress (PSS) are protected against lesion development. Different flow patterns may play differential roles in modulating protein phosphorylation in vascular endothelial cells (ECs) and hence atherogenesis. In this study, ECs isolated from inner curvature (OSS-dominant) and outer curvature (PSS-dominant) of aortic arch and thoracic aorta (PSS-dominant) of normal adult swine were subjected to phosphoprotein expression profiling. By using advanced bioinformatics-assisted phosphoproteomics, we found that vinculin is highly phosphorylated at serine residue 722 (Vin^{S722P}) in the inner curvature of aorta arch in comparison to the outer curvature and the thoracic aorta of porcine. This result was confirmed by using *en face* immunostaining of porcine and rat aorta arches *in vivo* and OSS/PSS-stimulated human aortic ECs (HAECs) in parallel-plate flow chamber *in vitro*. We further used fluorescence resonance energy transfer (FRET) technique (Figure 1) in the combination of confocal microscopy to elucidate the role of S722 phosphorylation in the activation of vinculin in HAECs. A full-length vinculin FRET probe containing EYFP in the end of head domain and ECFP at the COOH terminus of tail domain was constructed and transfected into HAECs, followed by exposure to different types of flows. The results showed that OSS, but not PSS, induces conformational changes of vinculin, with strong FRET signaling in HAECs, as compared with static control cells. Transfection of HAECs with a non-phosphorylatable Vin^{S722A} FRET probe, where the serine residue was replaced by alanine, resulted in inhibition in OSS-induced FRET signaling. In contrast, transfection with a phosphomimetic Vin^{S722E} FRET probe, where the serine residue was mutated to glutamic acid, exhibited strong FRET signal in PSS-stimulated HAECs. Our results using the combination of phosphorprotemics, FRET, and confocal microscopy demonstrated that S722 phosphorylation of vinculin is critical for its activation and conformational changes in ECs in response to disturbed flow, which may contributes to the development of atherosclerosis.

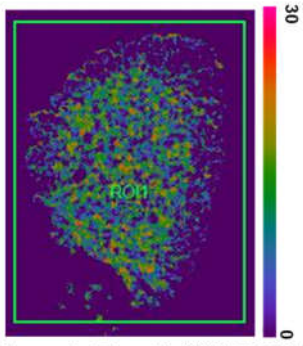

Figure 1: Vinculin^{S722P} FRET