The role of TIMAP in the regulation of protein phosphatase 1 and endothelial barrier function

by István Czikora, M.Sc.
Department of Medical Chemistry, Medical and Health Science Center University of Debrecen
Supervisor: Csilla Csortos, Ph.D
DOCTORAL SCHOOL OF MOLECULAR MEDICINE

Protein phosphatase 1 (PP1) regulates numerous cellular processes by dephosphorylating phospho-Ser/Thr residues of proteins. TIMAP protein (64 kDa), as a member of the MYPT family, is a putative regulatory subunit of the catalytic subunit of PP1 (PP1c). Our aim was to study the interaction between TIMAP and PP1c; and to study the effect of the phosphorylation of Ser333 and Ser337 side chains in TIMAP on this interaction. TIMAP is highly expressed in endothelial cells (EC) compared to other cell lines, and it localizes to the plasma membrane. Therefore we studied the role of TIMAP in the regulation of barrier function of human and bovine pulmonary artery endothelial cells.

Using several methods we provided evidence for specific interaction between TIMAP and PP1c; TIMAP binds preferentially the beta isoform of PP1c (K_a=1.8x10^6 M^{-1}). Thiophosphorylation of TIMAP by PKA or sequential thiophosphorylation by PKA and GSK3β only slightly modifies the association constant for the interaction of TIMAP with PP1c. However, non- and mono-thiophosphorylated forms of TIMAP inhibit PP1cβ activity, while the double-thiophosphorylated form does not affect the phosphatase activity with the utilized substrates.

To investigate the role of TIMAP in EC barrier regulation, we depleted TIMAP in HPAEC. We found that depletion of TIMAP attenuates the increases in transendothelial electrical resistance induced by the barrier protective agents (S1P and ATP) and enhances the effect of barrier-compromising agents (thrombin, nocodazole) demonstrating a barrier-protective role of TIMAP in EC. PKA activation by forskolin treatment of EC prevents thrombin evoked barrier dysfunction and ERM phosphorylation at the cell membrane. On the contrary in TIMAP depleted cells forskolin failed to affect the thrombin effect, and the ERM proteins remained phosphorylated. These data demonstrate that TIMAP is involved in the EC barrier protection as part of PKA-mediated ERM (ezrin-radixin-moesin) dephosphorylation. Using a specific GSK3β inhibitor we have shown that PKA activation is followed by GSK3β activation in bovine pulmonary EC and activation of both kinases is required for the rescuing effect of forskolin and protects the EC barrier function.

Keywords: TIMAP, Protein Phosphatase 1, ERM

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