BMC Pharmacology

Poster presentation

In vivo modulation of vasodilator stimulated phosphoprotein functions by phosphorylation

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from 2nd International Conference of cGMP Generators, Effectors and Therapeutic Implications
Potsdam, Germany, 10–12 June, 2005

Published: 16 June 2005

BMC Pharmacology 2005, **5**(Suppl 1):P4 doi:10.1186/1471-2210-5-S1-P4

The vasodilator-stimulated phosphoprotein (VASP) is a member of the Ena/VASP-family, regulating actin and cytoskeleton dynamics. Therefore, VASP is an important mediator in processes such as cell-cell adhesion, aggregation, and cellular motility in platelet, fibroblasts, and endothelial cells. The adaptor protein is located at the intersection of two major cyclic nucleotide-dependent signaling pathways, being a substrate of both cAMP- and cGMP-dependent kinase. *In vitro* it is well established that VASP is predominantly phosphorylated at Ser157 and Thr278 residues by cAMP- and at Ser239 by cGMP-dependent kinase, respectively. In contrast, the detailed cellular functions of distinct VASP phosphorylation(s) *in vivo* and kinases involved are controversial and are still a matter of debate.

In order to analyze the specific cellular role of different VASP phosphorylation patterns in living cells, we have generated a set of 20 VASP mutants. In these constructs, the phosphorylation sites Ser157, Ser239, and Thr278 were systematically exchanged to alanin and/or acidic amino acids, respectively, to mimic permanent non-phosphorylated or phosphorylated states. Using these mutants, we investigated subcellular localization in endothelial cells and effects of single, double or triple phosphorylated VASP on actin dynamics.

To specify the subcellular localization of the VASP mutants in the absence of endogenous VASP, we immortalized microvascular endothelial cells from VASP-deficient mice. Confocal laser scanning microscopy revealed that VASP mutants bearing acidic residues at position 157 localized to the cell membrane. Importantly, Ser157-phosphorylated endogenous VASP, probed by a phospho-specific antibody, was found in the same cellular compartment. In contrast, the membrane localization of overexpressed mutants in VASP$^{-/-}$ cells was independent of the phosphorylation state at Ser239 and/or Thr278. In line with these results, neither Ser239 nor Thr278 phosphorylation did alter the distribution of endogenous VASP. The effect of differential VASP phosphorylations on actin dynamics was scrutinized using a serum response factor-based reporter assay and FACS analysis of VASP-overexpressing cells. Phosphorylation at Ser239 and/or Thr278 increased the cellular amount of G-actin concomitant with an inhibition of F-actin fiber formation. On the other hand phoshorylations at Ser157 did not affect actin dynamics, indicating that merely phosphorylations at Ser239 and Thr278 but not Ser157, are important for actin polymerization.

Taken together, these results suggest that distinct VASP-phosphorylations have the potency to modulate the subcellular localization of the protein and the actin turnover *in vivo*, thereby linking cyclic nucleotide-dependent signaling pathways to the cytoskeleton.