

## **Functional Studies On Receptor-Type Protein Tyrosine Phosphatases Of The R3 Subgroup Using Bimolecular Fluorescence Complementation (BiFC) Assays**

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**Background:** The 21 human receptor-type tyrosine phosphatases (RPTPs) are characterised by distinct combinations of domains in their extracellular region; however, the functional role of this region has not been clearly defined for many RPTPs. Potential roles in ligand interaction, dimerisation, substrate specificity and cell-cell contacts have been reported (1). RPTPs of the R3 subgroup include vascular endothelial-protein tyrosine phosphatase (VE-PTP), density-enhanced phosphatase 1 (DEP-1), glomerular epithelial protein 1 (GLEPP-1) and stomach cancer-associated protein tyrosine phosphatase-1 (SAP-1), also termed as PTPRB, PTPRJ, PTPRO and PTPRH respectively. All these enzymes have a common structure consisting of multiple extracellular fibronectin type III-like domains, a transmembrane domain and a single cytoplasmic catalytic domain (2). VE-PTP is expressed in endothelial cells and is involved in regulation of endothelial cell contacts, vascular permeability and regulation of endothelial growth factors for angiogenesis. It was previously reported that VE-PTP associates with the adhesion molecule VE-Cadherin through their extracellular domains and plays an important role in the maintenance of the endothelial barrier function (3). GLEPP-1 was shown to associate with tropomyosin-related kinase C (TrkC) through which it exerts its regulatory action on axon guidance (2). One of the roles of SAP-1 is the maintenance of actin stress fibres and focal adhesions through the association with prominent focal adhesion-associated proteins p130<sup>Cas</sup> (4). DEP-1 plays a regulatory role in angiogenesis and inflammation through extracellular association with a proteoglycan Syndecan-2 with downstream  $\beta$ 1 integrin-mediated adhesion and cytoskeletal organisation (5).

**Objective:** The initial objective of this study was to define bimolecular fluorescence complementation (BiFC) method and to establish its selectivity as well as to validate the interaction between VE-PTP and VE-cadherin and then to explore possible dimerization of R3 RPTP members as a regulatory mechanism.

**Methods:** BiFC assay was validated using Jun/Fos and Jun/ $\Delta$ Fos fusion proteins. After attaining positive results, it was then used to visualise directly the interaction of VE-PTP with VE-cadherin in live cells. VE-PTP, VE-Cadherin and SPN (sialophorin, was used as a negative control in BiFC studies) fusion constructs containing full-length extracellular and transmembrane domains with either non-fluorescent N- or C-terminal fragments of the Venus yellow-fluorescent protein were successfully cloned and expressed.

**Results:** HEK293 cells were transfected with VE-PTP/VE-Cadherin, VE-PTP/VE-PTP and VE-PTP/SPN fusion pairs, all of which resulted in complementation of the fragments to form a fluorescent complex that was detected by confocal fluorescence microscopy. Expression of constructs was confirmed by immunofluorescence with antibodies to either the Myc or HA-tags.

**Conclusion:** Although the BiFC technique showed the interaction between the extracellular domains of VE-PTP and VE-cadherin and between VE-PTPs (demonstrating possible dimerization) it also showed positive interactions with the negative control SPN. Therefore, the obtained results are inconclusive and further work is required to validate specificity of the BiFC assay using better controls.

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