Investigation of interaction of receptor-type protein tyrosine phosphatases of the R3 subgroup with VE-Cadherin in live cells using bimolecular fluorescence complementation (BiFC) assays

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Receptor-like protein-tyrosine phosphatases (RPTPs) are involved in various aspects of cellular functions, such as proliferation, differentiation, survival, migration and metabolism. The 21 human 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. 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). 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. An endothelial specific adhesion molecule vascular endothelial (VE)-Cadherin has previously been identified as a substrate for VE-PTP in in-vitro experiments and co-immunoprecipitation studies. It has been reported that this interaction occurs via the extracellular domains of these molecules, independent of their catalytic domains, and that VE-PTP plays an important role in the maintenance of the endothelial barrier function. Here we examined the interaction of VE-PTP with VE-Cadherin via their extracellular domains by using the bimolecular fluorescence complementation (BiFC) assay in live cells and, in addition, we examined whether other members of the R3 RPTP subgroup also have the potential to interact with VE-Cadherin via their extracellular domains. An improved version of the bimolecular fluorescence complementation (BiFC) assay based on the Venus protein (a yellow fluorescent protein (YFP) variant) was used, which has an improved signal to noise and reduced background fluorescence. The extracellular domains of the R3 RPTP subgroup members were fused to N-terminal fragment of Venus-YFP and the extracellular part of the VE-Cadherin was fused to the C-terminal fragment of Venus-YFP. The split tagged proteins were transiently co-expressed in HEK293T cells. Controls consisted of a ΔVE-PTP construct, in which the 17th FNIII-like domain was removed, sialophorin, an unrelated membrane protein with a large extracellular domain and a membrane anchored C-terminal Venus-YFP fragment. Prior to visualisation the transfected cells were stained with CellMask™ Deep Red Plasma Membrane Stain for 90 minutes and images were obtained using a Leica confocal microscope. The BiFC assay has become increasingly popular in studies of protein-protein interactions in living cells due to its simplicity, ease of use and the fact that no sophisticated equipment is required to detect the fluorescence signal. However, this method has a major limitation of self-assembly between the two non-fluorescent yellow fluorescent protein fragments, contributing to false-positive fluorescence making data interpretation difficult. We found that qualitative measurements of fluorescence signal were not sufficient to interpret the data and draw any concrete conclusions as all the tested protein fusion pairs resulted in fluorescence signal, including the negative control. To obtain robust interpretations, quantitative comparisons were performed between the signal obtained with the protein combination of interest and the signal obtained with the negative control combination. Using ImageJ software the border of each cell was traced and the average pixel intensity of the selected region was calculated with instrument background subtracted. Our analysis in live cells revealed that: VE-PTP interacts with VE-cadherin via interactions involving the extracellular domain; deletion of the 17th FNIII-like domain of VE-PTP is not sufficient to disrupt this interaction; other R3-PTPs also have the potential to interact with VE-cadherin.