Antibody crossreactivity between the tumour suppressor PHLPP1 and the proto-oncogene β -catenin

During recent years, the PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) has received increasing attention as a tumour suppressor that functions by dephosphorylating and antagonizing the survival-promoting protein kinases AKT, PKC and S6K1 [1]. Previous studies reported that PHLPP1 co-localizes with the tumour suppressor Scribble at cell-cell contacts [2], its expression is lost in colorectal cancer tumours [3] and it correlates with expression of PTEN, a tumour suppressor phosphatase that functions as a negative regulator upstream from AKT [2,4]. We warn that the PHLPP1 antibodies used in these studies (Bethyl IHC-00382 and A300-660A) crossreact strongly with β -catenin, a proto-oncogene product known to localize to adherens junctions.

When probing Caco-2 whole cell lysates (WCLs) for PHLPP1 with commonly used commercial PHLPP1 antibodies (Bethyl IHC-00382 and A300-660A—different types of the same antibody), we observed an additional prominent immunoreactive band around 90 kDa (Fig 1C), which was still apparent on PHLPP1 immunoprecipitation using the same antibodies (Fig 1A). In contrast with full length PHLPP1, the 90 kDa band was not responsive to siRNA-mediated knockdown of PHLPP1 (Fig 1A,C), arguing against a PHLPP1 variant and suggesting it reflected antibody crossreactivity. To characterize further this putative crossreactivity, we used stable isotope labelling in cell culture and performed quantitative tandem mass spectrometry on PHLPP1 immunoprecipitates (Fig 1A). The oncogenic WNT-signalling component β -catenin was identified as the most prominent specifically purified protein—37.5% sequence coverage compared with 4.5% for PHLPP1; data not shown—having a reported molecular weight of 90 kDa. Indeed, β -catenin comigrated with the PHLPP1 antibody crossreactivity in WCL and PHLPP1

Fig 1 | PH domain leucine-rich repeat protein phosphatase 1 antibodies crossreact with β -catenin.

(A) Characterization of immunoreactivity of the PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) antibody Bethyl A300-660A indicates the presence of an additional prominent 90 kDa immunoreactivity as indicated with an asterisk. For quantitative mass spectrometry, Caco-2 cells were labelled with light (L, K0R0) or heavy (H, K6R10) isotopes for seven days and lysates were used for control (IgG) or A300-660A immunoprecipitation. Immunoprecipitates were pooled and processed for mass spectrometry (see text). (B) Crossreactivity of A300-660A with β -catenin. Caco-2 cells were transfected with control PHLPP1 or β -catenin siRNAs. Lysates were subjected to immunoprecipitation with control IgGs, PHLPP1 (A300-660A) or β -catenin (BD Biosciences, 610154) antibodies, and immunoblots were probed with the same antibodies. Bottom panel; dual colour visualization of the same blot, scanned by using the Odyssey dual colour imaging system with PHLPP1 in red and β -catenin in green as indicated. (C) PHLPP1 antibodies A300-660A (left panel) and IHC-00382 (right panel) show the same immunoreactivity in whole cell lysates. siRNA treatment of Caco-2 cells indicates that the 90 kDa crossreactivity (asterisk) is highly sensitive to β -catenin knockdown but not to PHLPP1 knockdown, with β -actin used as a loading control. (D) Immunostaining by using methanol fixation of PHLPP1 (IHC-00382) and β -catenin (BD Biosciences, 610154) in β -catenin-depleted Caco-2 cells. Asterisks indicate successfully depleted cells. Scale bar, 10 μ m. Immunohistochemical staining of PHLPP1, by using PHLPP1 (IHC-00382) and β -catenin (BD Biosciences, 610154) antibodies in colorectal tumours, shows plasma membrane staining (E) and nuclear staining (F).

immunoprecipitates (Fig 1B,C). Conversely, the PHLPP1 antibody strongly recognized a 90kDa species in β -catenin immunoprecipitates, (Fig 1B) further substantiating β -catenin as the underlying crossreactivity. To exclude alternative explanations, we performed siRNA-mediated knockdown of PHLPP1 and β -catenin. PHLPP1 antibody crossreactivity at 90kDa was strongly reduced after β -catenin knockdown without affecting full-length PHLPP1 levels (Fig 1C). β -catenin levels in PHLPP1 immunoprecipitates were not affected by PHLPP1 knockdown (Fig 1B). This argued against a prominent physiological direct association of PHLPP1 and β -catenin and thus explained the immunoreactivity (Fig 1A). Our observations were reproducible in other cell lines (HeLa and 293T), excluding the existence of Caco-2-specific artefacts.

These results raise concern for the potential contaminating effects of β -catenin, a known membrane-targeted factor, for localization studies of PHLPP1. Indeed, in confluent Caco-2 cells, we observed complete co-localization of PHLPP1 (IHC-00382) with β -catenin by immunofluorescence (Fig 1D). Strikingly, cells in which β -catenin levels had been depleted by siRNA (as indicated with an asterisk in Fig 1) lost PHLPP1 staining at cell-cell contact, whereas PHLPP1 staining remained prominent in cells that had poor β -catenin knockdown. Immunohistochemical staining of PHLPP1 and β -catenin within 5 μ m sections of each

other, from a tissue microarray of primary colorectal carcinomas ($n=742$; [5]), revealed a similar staining pattern with the two antibodies (Fig 1E) and, correspondingly, a non-physiologically strong correlation between PHLPP1 and β -catenin on membranes (p -value 2×10^{-64} ; Fig 1E) and in nuclear staining (p -value 1×10^{-14} ; Fig 1F).

Collectively, these results confirm the strong crossreactivity of PHLPP1 antibodies Bethyl IHC-00382 and A300-660A with β -catenin, making it impossible to conclude the intracellular localization of PHLPP1 using these antibodies. Although the cause of crossreactivity remains uncertain, we note that the PHLPP1-derived antigen used for immunization bears substantial sequential homology to the carboxyl terminus of β -catenin—74% similarity over eight amino acids.

As *in situ* analysis of the expression of potential biomarkers in patient tumours is used to correlate expression or localization with patient survival, it is important that the antibodies used in such studies are specific for the protein of interest. Given the localization of β -catenin and its implication in a wide variety of tumours, including those that are the focus of PHLPP1 studies, our observations strongly warn against the use of the antibodies Bethyl IHC-00382 and A300-660A in studies probing PHLPP1 function and inferring prognostic value to cellular PHLPP1 dynamics in tumour progression.

ACKNOWLEDGEMENTS

We thank Bernd Thiede and Magnus Arntzen (The Biotechnology Centre of Oslo, Norway) for help with mass spectrometry experiments.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO reports (2013) **14**, 10–11; published online 11 December 2012; doi:10.1038/embor.2012.188