BioFeedback

[Letter to the editor]

Commercial Cdk1 antibodies recognize the centrosomal protein Cep152

The centrosome is a highly organized cellular organelle that functions as the main microtubule-organizing center (MTOC) of animal cells and is crucial for fundamental cellular processes (1). Proteomic analyses have revealed a network of interactions among centrosomal proteins (2,3). We report here that two widely used commercial mouse monoclonal antibodies against the master cell cycle regulator Cdk1 (sc-54, Santa Cruz Biotechnology, Dallas, TX; ab18, Abcam, Cambridge, United Kingdom) cross-react with the centrosomal protein Cep152. Our results provide an explanation for previous conflicting reports on the localization of Cdk1 and also question the reported centrosomal recruitment of Cdk1 by Cep63.

Recently, we reported the existence of a Cep57-Cep63-Cep152 centrosomal complex, in which Cep63 and Cep152 exhibit a strong mutual dependency for their localization at centrosomes (4). Given that Cep63 has been reported to be required for the presence of the master cell cycle regulator Cdk1 at centrosomes (5,6), we expected that centrosomal Cdk1 localization should also depend on Cep152 function. To test this hypothesis, we carried out pull-down experiments, performing Western blot analysis of the fusion proteins purified from HEK293 cells expressing SNAP-Cep152, SNAP-Cep63, SNAP-Cep57, or Cdk1-SNAP. SNAP-tag is a 20 kD

protein tag that can be covalently labeled (e.g. with fluorescent dyes), thus permitting detection of the fused proteins in vitro and in vivo (7–9). We probed the membrane with the mouse monoclonal anti-Cdk1 antibody sc-54 that was utilized in the study reporting the dependency of centrosomal Cdk1 on Cep63 function (5). This antibody has also been used in over 200 published studies (www.scbt.com/datasheet-54-cdc2-p34-17-antibody.html).

Surprisingly, we found that sc-54 recognized not only Cdk1, but also Cep152 (Figure 1). To clarify this issue, we purchased four other commercial anti-Cdk1 antibodies that were raised against other Cdk1 immunogens and repeated the Western blot analysis (Table 1, Figure 1, Supplementary Figure S3B). We found that the mouse monoclonal anti-Cdk1 antibody ab18 similarly cross-reacted with Cep152, whereas the rabbit polyclonal HPA003387 antibody or mouse monoclonal antibodies ab8040 and 610038 did not (Figure 1).

The observed cross-reactivity of some anti-Cdk1 antibodies does not pose a significant problem in simple Western blotting experiments because of the size difference between Cdk1 and Cep152, but it could potentially cause misleading results in immunofluorescence and pull-down experiments. We therefore set out to perform immunofluorescence with all five antibodies to determine whether the cross-reactivity observed by Western blot also applies to this experi-

mental approach, which was previously used to report the reliance of centrosomal Cdk1 on Cep63 function (5). We fixed control cells and cells depleted of Cdk1 or Cep152, using two distinct siRNAs/siLNAs corresponding to each protein. The resulting samples were then stained with anti-Cdk1 antibodies (one antibody per sample), along with anti- γ -tubulin antibodies to mark centrosomes. In each case, we quantified the resulting centrosomal signal intensity in cells in late G2/early mitosis relative to the γ -tubulin signal; the outcome of these experiments is summarized in Table 1, and more detailed results are listed in Supplementary Table S1. First, we found that both sc-54 and ab18 antibodies labeled centrosomes in control conditions throughout the cell cycle, as reported previously for sc-54 (10). These results contrast with earlier studies using PSTAIR and CTR453 antibodies, which led to the conclusion that Cdk1 localizes to centrosomes solely in late G2/early mitosis (11). In accordance with the latter result, we found that in control conditions the HPA003387, ab8040, and 610038 antibodies indeed significantly labeled centrosomes solely during late G2/early mitosis (Supplementary Figure S1A). Second, we found that the centrosomal signal of the sc-54 and ab18 antibodies decreased substantially upon depletion of Cep152 but not upon depletion of Cdk1 (Table 1, Supplementary Table S1, Supplementary Figure S1B). Conversely, we found

Table 1. Properties of tested Cdk1 antibodies

Cdk1 Antibody (Company)	Species and type	Signal intensity after depletion of:			Present solely in late G2/mitosis	Recognizes SNAP-Cep152 on WB
		Cdk 1	Cep152	Cep63		
sc-54 (Santa-Cruz)	Mouse monoclonal	High	Low	Low	NO	YES
ab18 (Abcam)	Mouse monoclonal	High	Low	ND	NO	YES
HPA003387* (Sigma-Aldrich)	Rabbit polyclonal	Low	High	ND	YES	NO
ab8040* (Abcam)	Mouse monoclonal	Low	High	High	YES	NO
610038* (BD Biosciences)	Mouse monoclonal	Low	High	High	YES	NO

A minimum of 13 cells (26 centrosomes) were analyzed in each case. * indicates that the centrosomal signal was measured only in cells in late G2/early mitosis. High signal intensity corresponds to normalized median values > 0.5; low signal intensity corresponds to normalized median values < 0.5 (see Supplementary Table S1). ND: not determined.

Figure 1. Characterization of Cdk1 antibodies using Western blotting. Western blots with five different Cdk1 antibodies are presented to illustrate the reactivity of antibodies to SNAP-tag fusion proteins purified from HEK 293 cells. The upper row shows Western blot membranes scanned for SNAP-tag fluorescence labeled with SNAP-Surface 488 substrate. The lower row shows Western blot membranes scanned for Cdk1 antibody fluorescence detected with an appropriate secondary antibody labeled with Alexa647. Note that the 610038 (BD Biosciences) antibody recognizes only the free Cdk1 C-terminus and thus detects SNAP-Cdk1 but not Cdk1-SNAP. Black arrows indicate SNAP-tagged Cdk1. Gray arrows indicate SNAP-Cep152. M indicates the PageRuler prestained protein ladder; numbers above PageRuler bands indicate molecular weight in kDa.

little or no change in the centrosomal signals for the HPA003387, ab8040, or 610038 antibodies upon depletion of Cep152, but a substantial decrease was observed upon depletion of Cdk1 (Table 1, Supplementary Table S1). Analogous results were obtained when Cep63 was depleted by RNA interference (Supplementary Figure S1B), as expected given that Cep63 and Cep152 are mutually dependent for their centrosomal localization (4).

Next, we conducted confocal imaging of U2OS cells to precisely localize the centrosomal signal of anti-Cdk1 antibodies relative to C-Nap1, which marks the proximal end of the centriole, or Cep152, which is present in a ring just above the proximal end (6,12–15). The confocal microscopy experiments established that both sc-54 and 610038 antibodies labeled the proximal end of the centriole (Supplementary Figure S2A and B). Interestingly, however, we found that the distribution of the sc-54 signal was different from that

of the 610038 signal, with the former matching the localization of the Cep152 ring perfectly (Supplementary Figure S2C and D). To confirm this, we generated a U2OS cell line expressing a Cdk1-SNAP fusion protein. Immunofluorescence experiments showed that the Cdk1-SNAP signal resembled the staining obtained with the 610038 antibody and not the sc-54 antibody (Supplementary Figure S2E and F). To investigate whether the distribution of Cep152 and Cdk1 at centrosomes may differ, we performed STED (stimulated emission depletion) microscopy. In accordance with previous observations (4), super-resolution microscopy showed that Cep152 was present at the proximal end of the parental centriole in a ring-like structure 367 nm in diameter on average (Supplementary Figure S3A). By contrast, we found that the 610038 antibody signal was positioned in a much more diffuse manner, most likely in the pericentriolar material (PCM), and could not be resolved as a ring by

STED microscopy (Supplementary Figure S3A).

Finally, we found that there may be a simple explanation for the observed cross-reactivity between Cdk1 and Cep152 using the sc-54 and the ab18 antibodies. Indeed, these antibodies were raised against partially overlapping immunogens of Cdk1, which exhibit some degree of similarity with the amino acid sequence of Cep152 (Supplementary Figure S3B and C).

In conclusion, our observations indicate that both sc-54 and ab18 antibodies recognize not only Cdk1 but also Cep152 in Western blot and immunofluorescence assays. By contrast, HPA003387, ab8040, and 610038 appear to recognize Cdk1 specifically, and demonstrate that Cdk1 localizes most likely in the PCM of centrosomes in a cell-cycle dependent manner. Our work also serves as a reminder that numerous independent control experiments are needed to verify antibody specificity in immunofluorescence and other antibody-based experiments (16).

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Competing interests

The authors declare no competing interests.

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