RB798, RB799, RB800, RB801, RB802, RB803, RB804, RB805, RB806 and RB807 antibodies recognize a human Aurora kinase B (AURKB) phosphorylated peptide by ELISA

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Abstract

The recombinant antibodies RB798, RB799, RB800, RB801, RB802, RB803, RB804, RB805, RB806, and RB807 were tested by ELISA for their binding to a synthetic phosphorylated peptide from the human Aurora kinase B (AurKB) protein. Antibodies RB805 and RB806 detected both the phosphorylated and non-phosphorylated peptides, whereas the remaining antibodies selectively recognized the phosphorylated peptide.

Introduction

Aurora kinase B (AurKB) (UniProt #Q96GD4) is a key regulator of the attachment between kinetochores and spindle microtubules (Andrews *et al*., 2004). Here we describe the ability of eight recombinant antibodies (RB798, RB799, RB800, RB801, RB802, RB803, RB804, and RB807) to selectively detect by ELISA a synthetic phosphorylated peptide from the AurKB protein. Antibodies RB805 and RB806 recognize both the phosphorylated and the non-phosphorylated forms of the AurKB peptide.

Materials & Methods

Antibodies: ABCD_RB798, ABCD_RB799, ABCD_RB800, ABCD_RB801, ABCD_RB802, ABCD_RB803, ABCD_RB804, ABCD_RB805, ABCD_RB806 and ABCD_RB807 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding scFv portion fused to a human IgG1 Fc (HRB798, HRB799, HRB800, HRB801, HRB802, HRB803, HRB804, HRB805, HRB806 and HRB807). HEK293 suspension cells (growing in HEK TF medium, Xell #861-0001, supplemented with 0.1% Pluronic F68, Sigma #P1300) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~60 to 110 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to residues 223 to 233 of the AurKB protein phosphorylated on threonine 232 (pT232) (VHAPSLRRKpTM). The non-phosphorylated version of the same N-biotinylated peptide (T232) (VHAPSLRRKTM) was used as used as a negative control for the ELISA on figure 1A. An irrelevant biotinylated peptide (GVENKTPAVTpSDITYGV) from a scrambled sequence of high mobility group protein B1 (UniProt #P09429) was used as a negative control for the ELISA on figure 1B.

Protocol: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of HRB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-human IgG (BioRad #1721050, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Results

Antibodies RB798, RB799, RB800, RB801, RB802, RB803, RB804 and RB807 bound in a concentration-dependent manner to the phosphorylated AurKB peptide against which they were selected, but not to the non-phosphorylated peptide (Fig. 1A). RB805 and RB806 recognized both the phosphorylated and non-phosphorylated AurKB peptides but not the irrelevant peptide used as control (fig. 1B). Although these antibodies recognize specifically the AurKB peptides by ELISA, their ability to bind the full-length protein should be determined in future experiments.

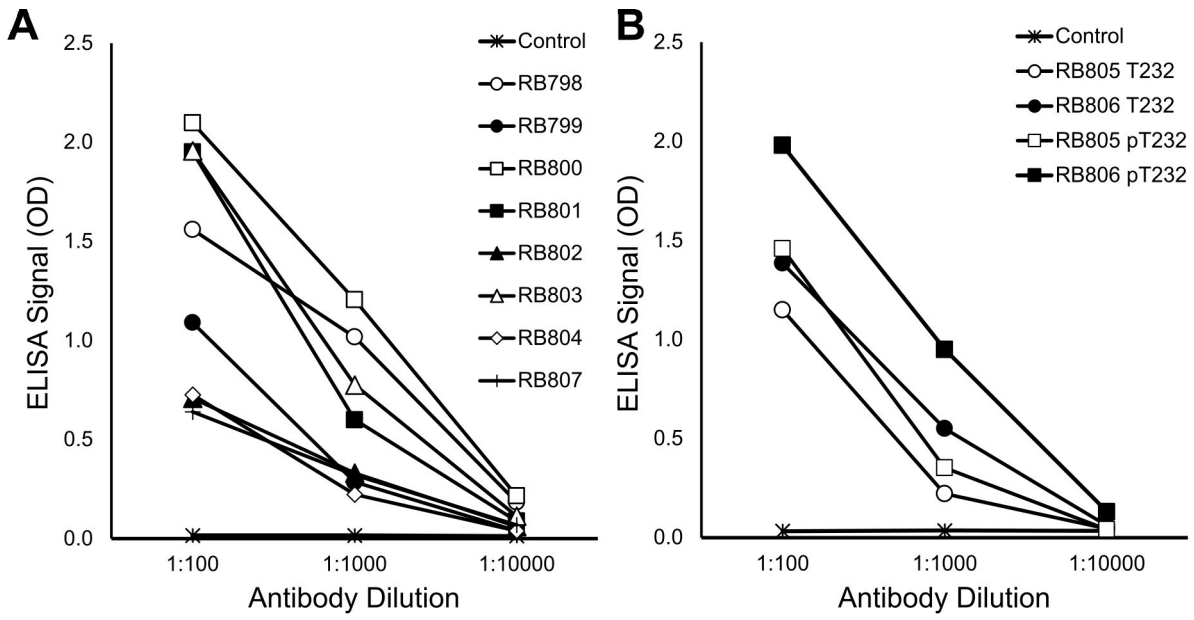

Fig. 1. Specific binding of HRB antibodies to the target AurKB peptide, as detected by ELISA. (**A)** Binding of antibodies HRB798 to HRB804 and HRB807 to the phosphorylated AurKB peptide. RB802 and RB807 curves are difficult to distinguish because they are almost superimposed. 'Control' indicates the binding of HRB798 to the non-phosphorylated peptide (all other control curves were superimposed). (**B)** Binding of HRB805 and HRB806 antibodies to the phosphorylated (pT232) and non-phosphorylated AurKB peptide (T232). 'Control' indicates the binding of HRB805 to the irrelevant peptide (all other control curves were superimposed).

References

Andrews PD, Ovechkina Y, Morrice N, Wagenbach M, Duncan K, Wordeman L, Swedlow JR. Aurora B regulates MCAK at the mitotic centromere. Dev Cell. 2004 Feb;6(2):253-68. PMID: 14960279.

Conflict of interest

Philippe Hammel is a cofounder and a shareholder of ABCD Antibodies SA.