RB393 and RB394 antibodies recognize a peptide from the Dictyostelium AplA protein by ELISA

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Abstract

The recombinant antibodies RB393 and RB394 detect by ELISA a synthetic peptide from the *D. discoideum* AplA protein.

Introduction

AplA (Amoeba Saposin A, DDB_G0284043, UniProt # Q54Q68) is a member of the saposin family of proteins (IPR008373). Saposins aid in the activation of hydrolase enzymes involved in the breakdown of sphingolipids (Rorman *et al.,* 1989). Here we describe the ability of two recombinant antibodies (RB393 and RB394) to detect by ELISA a synthetic biotinylated peptide from the AplA protein.

Materials & Methods

Antibodies: ABCD_RB393 and ABCD_RB394, antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies) and produced as mini-antibodies with the antigen-binding scFv portion fused to a mouse IgG Fc (MRB393 and MRB394). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco#12338) were transiently transfected with the vector coding for the scFv of each antibody. Supernatants (MRB393: 90µg/mL, MRB394: 60µg/mL) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide of 17 amino acids corresponding to residues 302-318 of the AplA protein (PAPTPTSTPSTIKIDVN, **AplA.a**). As a negative control, a N-biotinylated peptide corresponding to residues 370-388 (EYLEFAVTQLEAKFNPTEI, **AplA.b**) was used.

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 MRB 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-mouse IgG (Bio-Rad #170-6516, 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 MRB393 and MRB394 bound the AplA.a peptide against which they were raised, but not to the negative control AplA.b peptide (Fig. 1). Although these two antibodies recognize specifically the AplA.a peptide in ELISA, their ability to bind the full-length protein should be determined in future experiments.

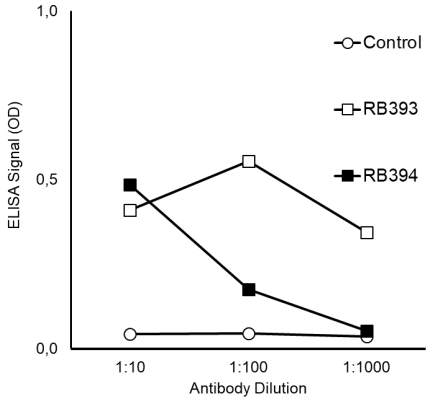

Fig. 1. Specific binding of MRB antibodies to the target AplA.a peptide, as detected by ELISA. 'Control' indicates the binding of MRB394 to the negative control AplA.b peptide (all other control curves were superimposed).

References

Rorman EG, Grabowski GA. Molecular cloning of a human co-beta-glucosidase cDNA: evidence that four sphingolipid hydrolase activator proteins are encoded by single genes in humans and rats. Genomics. 1989 Oct;5(3):486-92. PMID: 2515150.

Conflict of interest

The authors declare no conflict of interest.