RB045 and RB048 antibodies recognize a peptide of the D. discoideum SibA protein by western blot

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Abstract

Recombinant antibodies RB045 and RB048 detect by western blot a peptide of the *Dictyostelium discoideum* SibA protein fused to a GST protein.

Introduction

SibA (Integrin beta-like protein A, DDB_G0287363, UniProt #Q54KF7) is protein similar to metazoan integrin beta chains, involved in cell adhesion and phagocytosis in *D. discoideum* (Cornillon *et al*., 2006). Here we describe the ability of the RB045 and RB048 antibodies to detect by western blot a fragment of the SibA protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RB045 and ABCD_RB048 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc (MRB045 and MRB048). HeLa cells (growing in DMEM GlutaMAX™ (Gibco, #31966) supplemented with 8% Fetal Bovine Serum (Gibco, #10270)) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~1 mg/L) were collected after 4 days.

Antigen: The antibodies were originally raised against a GST protein fused to the last 46 cytosolic residues (KKSAPPTDAFFGEGAFADGAVSTNPMYEESGRSAINPLYEASSENL) of the SibA protein. This chimeric GST-SibA protein was used as antigen for detection. GST was used as a negative control.

Protocol: Expression of the GST-SibA recombinant protein was induced in *E. coli* bacteria growing exponentially (OD_{600}, 0.5) at 37°C (in 50 ml of Luria-Bertani (LB) medium containing 20% glucose and 100 µM ampicillin) by addition of 1.5 mM IPTG. After 3 h, bacteria were pelleted and resuspended in lysis buffer (4 ml of PBS + 1% Triton X100 + aprotinin 10 µg/ml + leupeptin 20 µg/ml + iodoacetamide 1.8 mg/ ml + PMSF 18 µg/ml) and lysed by sonication. GST was purified on glutathione-coupled sepharose 4 Fast Flow beads (GE Healthcare Life Sciences #17-5132-01), then eluted in 500 µl of reducing sample buffer (20.6% (w/v) sucrose, 100 mM Tris pH 6.8, 10 mM EDTA, 0.1% (w/v) bromophenol blue, 4% (w/v) SDS, 6% (v/v) β-mercaptoethanol). 15 µL of each sample was migrated (200 V, 30 min) in a 12% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1043), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked overnight at 4 °C in PBS containing 0.1% (v/v) Tween20 and 5% (w/v) milk, and washed three times (5 minutes) in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with each of the tested antibodies (undiluted), for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse (Biorad #170-6516, dilution 1:3000) for 1h at room temperature, and washed three times (5 minutes) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) using a PXi-4 gel imaging systems (Syngene).

Results

RB045 and RB048 antibodies specifically recognize the GST-SibA fusion protein (~31 kDa), as well as a probable partial degradation product at ~18kDa (for RB045). The antibodies do not bind the GST negative control (Fig. 1). The antigen used is a short cytosolic domain. It presumably does not fold into a complex structure, nor contains post-translational modifications. Accordingly, it is likely that the antibodies will also recognize the full-length protein. Further experiments will be necessary to determine if this is the case, and in which experimental procedures these antibodies can be used.

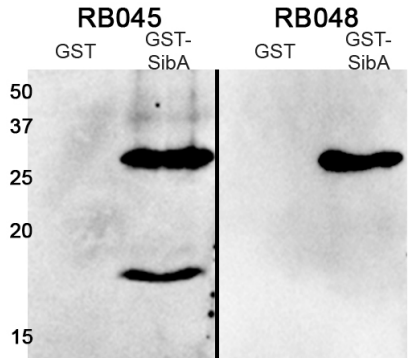

Fig. 1. Specific binding of RB045 and RB048 antibodies to the GST-SibA protein (predicted molecular mass ~31 kDa).

References

Blanc C, Zufferey M, Cosson P. Use of in vivo biotinylated GST fusion proteins to select recombinant antibodies. ALTEX. 2014;31(1):37-42. PMID:24100547

Cornillon S, Gebbie L, Benghezal M, *et al*. An adhesion molecule in free-living *Dictyostelium* amoebae with integrin beta features. EMBO Rep. 2006; 7(6):617-21. PMID:16699495

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. Nucleic Acids Res. 2020; 48(D1):D261-D264. PMID:31410491