RB155, RB156 and RB189 antibodies recognize a peptide from the D. discoideum *Tsg101 protein by ELISA*

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Abstract

The recombinant antibodies RB155, RB156 and RB189 detect by ELISA a fragment of the *D. discoideum* Tsg101 fused to a GST protein.

Introduction

The *Dictyostelium discoideum* Tsg101 (Tumor Susceptibility Gene 101, DDB_G0286797, UniProt #Q54LJ3) is a component of the ESCRT-I complex, potentially involved in vesicular trafficking and sorting in endosomal compartments (Blanc *et al.*, 2009). Here we describe the ability of three recombinant antibodies (RB155, RB156 and RB189) to detect by ELISA a fragment of the Tsg101 protein fused to a GST protein.

Materials & Methods

Antibodies: ABCD_RB155, ABCD_RB156 and ABCD_RB189 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 (MRB155, MRB156 and MRB189). HEK293 suspension cells (growing in DMEM, Gibco #11960044 supplied with 8%FBS) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~1-5 mg/l) were collected after 5 days.

Antigen: The antibodies were originally raised against a GST protein fused to the 20 first residues of Tsg101 (`MYGHHGYPMHAHQQQMVNPT`). This chimeric GST-Tsg101 was used as antigen for ELISA detection. GST was used as negative control.

Protocol: The whole procedure was carried out at room temperature. Bacterial lysates containing GST proteins were incubated in a glutathione-coated 96-well plate (Pierce #15240) 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 MRB155, MRB156 and MRB189 bound in a concentration-dependent manner to the GST-Tsg101 antigen, but not to the GST negative control (Fig. 1).

Note that this antigen only encompasses a small portion of the Tsg101 protein, and that it is presumably not properly folded. Further experiments will be necessary to determine if and in what conditions these antibodies recognize the full Tsg101 protein.

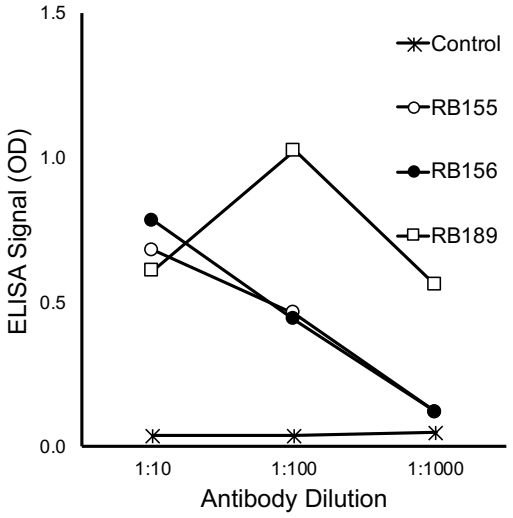

Fig. 1. Specific binding of MRB antibodies to the target GST-Tsg101 protein, but not to GST (shown only for MRB155; MRB156 and MRB189 background curves are superimposed), as detected by ELISA.

References

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Conflict of interest

The authors declare no conflict of interest.