The AJ156 antibody recognizes the Dictyostelium Talin A protein by Western blot

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Abstract

The AJ156 antibody, derived from the 169-477-5 hybridoma, detects by Western blot the full-length Talin A protein from *Dictyostelium discoideum*.

Introduction

Talin A (TalA, Filopodin, DDB_G0290481, UniProt #P0CE95) is a cytoskeletal protein, present mostly in the tips of filopods of *D. discoideum*, recognized by the 169-477-5 monoclonal antibody (Kreitmeier *et al*., 1995). Here we describe the ability of the AJ156 antibody, a single chain fragment (scFv) derived from the 169-477-5 hybridoma, to detect the full-length TalA protein by Western blot.

Materials & Methods

Antibodies: ABCD_AJ156 antibody (ABCD nomenclature, web.expasy.org/abcd/) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibody with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions joined by a peptide linker $(GGGGS)_3$. The sequencing of the 169-477-5 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (~50 mg/L) were collected after 4 days.

Antigen: $5x10^6$ *D. discoideum* DH1 cells, cultivated in HL5 medium, were used to detect the full-length TalA protein.

Protocol: Cells were pelleted and resuspended in 100 µL of 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 was added for reducing condition; reduced samples were also boiled for 15 min at 95 ºC). 10 µL of each sample ($5x10^5$ cells) was migrated (200 V, 120 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, #M00655), and transferred to a nitrocellulose membrane using a dry transfer system for 7 minutes (iBlot gel transfer device, Invitrogen #IB23001). The membranes were blocked during 1 hour in PBS containing 0.2% (v/v) Tween20 and 5% (w/v) milk, and washed once for 15 minutes in PBS + 0.2% (v/v) Tween20. The membranes were then incubated overnight with either the 169-477-5 monoclonal or the AJ156 scFv antibody (diluted 1:10 in PBS-Tween). The membranes were then washed three times for 15 minutes and incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad #170-6516, dilution 1:1000) and washed three times for 15 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Immobilon Classico Western HRP substrate, Millipore #WBLUC0500) using a PXi-4 gel imaging systems (Syngene).

Results

Similarly to the original 169-477-5 hybridoma, the AJ156 antibody specifically recognizes the TalA protein, detecting a single band around 220 kDa (Fig. 1) (Kreitmeier *et al*., 1995).

References

Kreitmeier M, Gerisch G, Heizer C, Müller-Taubenberger A. A talin homologue of Dictyostelium rapidly assembles at the leading edge of cells in response to chemoattractant. J Cell Biol. 1995; 129(1):179-88. PMID:7698984

Conflict of interest

Wanessa Cristina Lima is an editor of the Antibody Reports journal.

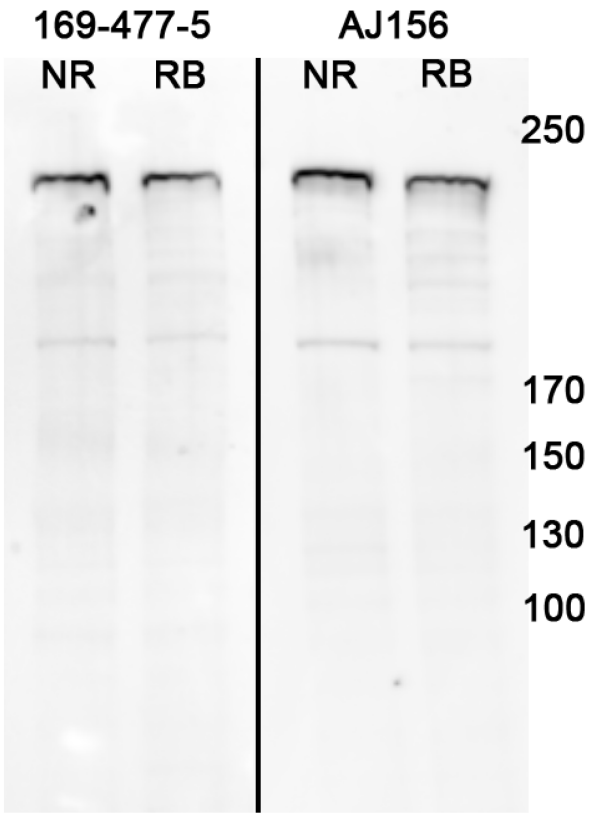

Fig. 1. The 169-477-5 hybridoma and the AJ156 scFv antibodies specifically recognize the TalA protein (although the predicted molecular mass of TalA is 269 kDa, the band detected is around 220 kDa, as also observed in the original description of the hybridoma by Kreitmeier *et al.*, 1995) (NR: non-reducing conditions; RB: reducing and boiled conditions).