The AJ154 antibody recognizes the Dictyostelium p80 protein by Western blot

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Abstract

The AJ154 antibody, derived from the H161 hybridoma, detects by Western blot the full-length p80 protein from *Dictyostelium discoideum*.

Introduction

The p80 protein (DDB_G0287297, UniProt #Q7YXD4) is a widely-used marker for endosomal compartments in *D. discoideum*, recognized by the H161 monoclonal antibody (Ravanel *et al*., 2001). Here we describe the ability of the AJ154 antibody, a single chain fragment (scFv) derived from the H161 hybridoma, to detect the full-length p80 protein by Western blot.

Materials & Methods

Antibodies: ABCD_AJ154 antibody (ABCD nomenclature, https://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 three different Fc moieties: mouse IgG2A, human IgG1 and rabbit IgG. 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 H161 hybridoma was performed by the Geneva Antibody Facility. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~50 mg/L) were collected after 5 days.

Antigen: *D. discoideum* DH1 (WT) cells were used to detect the full-length p80 protein.

Protocol: 10^5 and 10^4 *D. discoideum* cells were pelleted and resuspended in 20 µL of non-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 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1086), and transferred to a nitrocellulose membrane using a dry transfer system for 10 minutes (iBlot gel transfer device, Invitrogen #IB1001EU). The membranes were blocked during 1 hour in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk, and washed once for 15 minutes in PBS + 0.1% (v/v) Tween20. The membranes were then incubated with the original mouse hybridoma H161 supernatant (dilution 1:20 in PBS-Tween) or with each of the reformatted scFv antibodies (dilution 1:100 in PBS-Tween), overnight at 4 °C, then washed three times for 15 minutes in PBS-Tween. The membranes were then incubated with horseradish peroxidase-coupled goat anti-mouse, anti-human or anti-rabbit IgG (Biorad #170-6516, Biorad #172-1050, and Sigma #A8275 respectively, dilution 1:3000) and washed three times for 15 minutes and once for 5 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 H161 hybridoma, the AJ154 antibody specifically recognizes the p80 protein in *D. discoideum* cells (Fig. 1).

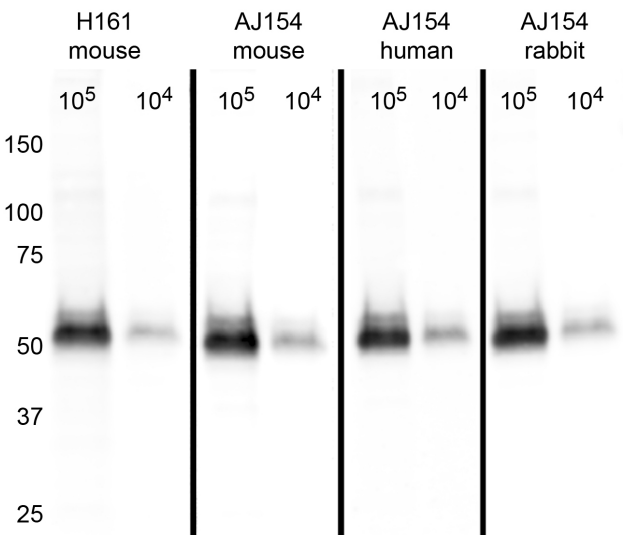

Fig. 1. Specific binding of H161 hybridoma and AJ154 antibody to the p80 protein (predicted molecular mass ~58 kDa).

References

Ravanel K, de Chassey B, Cornillon S, *et al*. Membrane sorting in the endocytic and phagocytic pathway of Dictyostelium discoideum. Eur J Cell Biol. 2001;80(12):754-64. PMID:11831389

Conflict of interest

The authors declare no conflict of interest.