The AJ520 antibody recognizes the Dictyostelium vacuolar H^+-ATPase subunit A by Western blot

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Abstract

The AJ520 antibody, derived from the 221-35-2 hybridoma, detects by Western blot the full-length vacuolar H^+-ATPase subunit A from *Dictyostelium discoideum*.

Introduction

The vacuolar H^+-ATPase subunit A (VatA, DDB_G0287127, UniProt #P54647) is a membrane protein present in the contractile vacuole and endosomal compartments in *D. discoideum*, recognized by the 221-35-2 monoclonal antibody (Jenne *et al*., 1998; Neuhaus *et al*., 1998). Here we describe the ability of the AJ520 antibody, a single chain fragment (scFv) derived from the 221-35-2 hybridoma, to detect the full-length VatA protein by Western blot.

Materials & Methods

Antibodies: ABCD_AJ520 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 221-35-2 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 protein.

Protocol: Cells were pelleted and resuspended in 200 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). 20 µL of each sample was migrated (200 V, 30 min) in a 4-15% acrylamide gel (Mini-PROTEAN® TGX™ Precast Gel, Biorad #456-1083), 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 0.1% (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 during 2 hours with either the 221-35-2 monoclonal or the AJ520 scFv antibody (diluted 1:3 or 1:10 in PBS-Tween, respectively). The membranes were then washed three times and incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad#170-6516, dilution 1:3000) and washed twice for 15 minutes and once for 5 minutes in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

Similarly to the original 221-35-2 hybridoma, the AJ520 antibody specifically recognizes the VatA protein, detecting a single band around 68 kDa (Fig. 1) (Fok *et al*., 1993; Neuhaus *et al*., 1998).

References

Fok AK, Clarke M, Ma L, Allen RD. Vacuolar H(+)-ATPase of Dictyostelium discoideum. A monoclonal antibody study. J Cell Sci. 1993;106:1103-13. PMID: 8126094

Jenne N, Rauchenberger R, Hacker U, Kast T, Maniak M. Targeted gene disruption reveals a role for vacuolin B in the late endocytic pathway and exocytosis. J Cell Sci. 1998;111:61-70. PMID:9394012

Neuhaus EM, Horstmann H, Almers W, Maniak M, Soldati T. Ethane-freezing/methanol-fixation of cell monolayers: a procedure for improved preservation of structure and antigenicity for light and electron microscopies. J Struct Biol. 1998;121(3):326-42. PMID:9704504

Conflict of interest

The authors declare no conflict of interest.

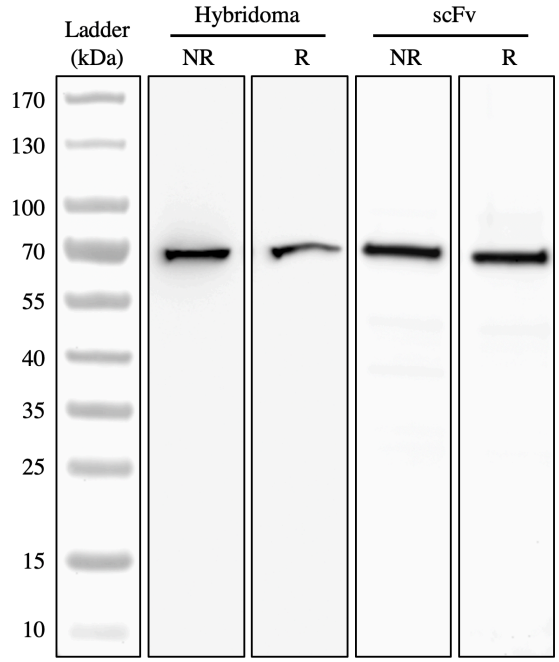

Fig. 1. The 221-35-2 hybridoma and the AJ520 scFv antibodies specifically recognize the VatA protein (predicted molecular mass ~68 kDa) (NR: non-reducing conditions; R: reducing conditions).