The AK421 antibody recognizes the Dictyostelium mitochondrial porin by immunofluorescence

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Abstract

The AK421 antibody, derived from the 70-100-1 hybridoma, detects by immunofluorescence the mitochondrial porin from *Dictyostelium discoideum*.

Introduction

The mitochondrial outer membrane porin (porA, DDB_G0271848, UniProt #Q01501), also known as voltage-dependent anion-selective channel (VDAC), is a transmembrane protein that acts as a permeability channel for hydrophilic compounds (Troll *et al*., 1992). Here we describe the ability of the AK421 antibody, a single chain fragment (scFv) derived from the 70-100-1 hybridoma, to label mitochondria by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AK421 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 rabbit IgG 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 70-100-1 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 (~20 mg/L) were collected after 4 days.

Antigen: $5x10^5$ *D. discoideum* DH1 cells, sedimented on a 22x22 mm glass coverslip (Menzel-Gläser) for 1 h at room temperature in HL5 medium, were used to detect the full-length protein.

Protocol: Cells were fixed with HL5 + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, then washed once in PBS for 5 min. Cells were then permeabilized in methanol at -20 °C for 2 min, washed once (5 min) with PBS, and blocked for 30 min with PBS + 0.2% (w/v) BSA (PBS-BSA). Cells were then incubated for 30 min with the original mouse hybridoma 70-100-1 supernatant (dilution 1:3 in PBS-BSA) and with the reformatted AK421 scFv antibody (dilution 1:10 in PBS-BSA). After 3 washes (5, 5, 15 min) with PBS-BSA, cells were incubated for 45 min with secondary goat anti-mouse IgG conjugated to AlexaFluor-488 (hybridoma) and goat anti-rabbit IgG conjugated to AlexaFluor-647 (scFv) (1:300, Molecular Probes #A11029 and #A21245, respectively). After 3 washes (5, 5, 15 min) with PBS-BSA and one wash (5 min) with PBS, coverslips were mounted on slides (Menzel-Gläser, 76x26 mm) with Möwiol (Hoechst) + 2.5% (w/v) DABCO (Fluka, #33480). Pictures were taken using a Zeiss LSM700 confocal microscope, with a 63x Neofluar oil immersion objective.

Results

In agreement with the original description of the 70-100-1 hybridoma (Troll *et al*., 1992), the AK421 antibody labels mitochondria (Fig. 1). The staining with both antibodies appears almost indistinguishable (Fig. 1).

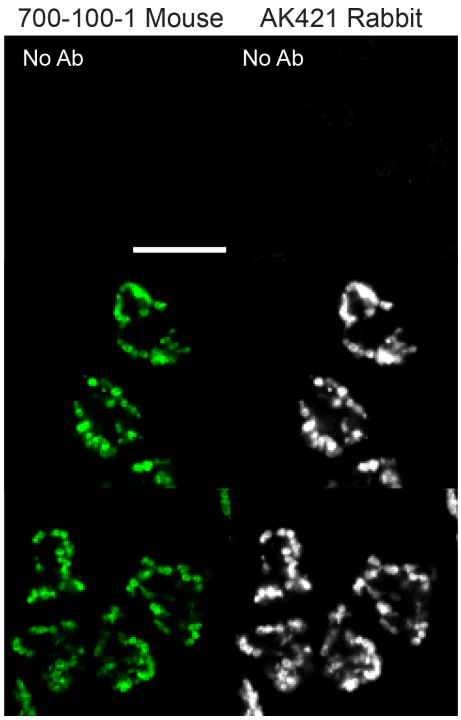

Fig. 1. The 700-100-1 hybridoma and the AK421 scFv label mitochondria in *Dictyostelium* cells. A double immunofluorescence staining with 700-100-1 and AK421 was performed. No labelling was seen when the primary antibodies were omitted (No Ab). Scale bar: 10 µm.

References

Troll H, Malchow D, Müller-Taubenberger A, *et al*. Purification, functional characterization, and cDNA sequencing of mitochondrial porin from Dictyostelium discoideum. J Biol Chem. 1992; 267:21072-9. PMID: 1328220

Conflict of interest

The authors declare no conflict of interest.