The AK423 antibody recognizes Dictyostelium actin network by immunofluorescence

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Abstract

The AK423 antibody, derived from the 224-236-1 hybridoma, detects by immunofluorescence the actin network of *Dictyostelium discoideum*.

Introduction

Actin is one of the most abundant proteins in eukaryotic cells, and a major structural component of the cytoskeleton. The major actin protein from *Dictyostelium* (UniProt #P07830) is encoded by 17 identical genes. Here we describe the ability of the AK423 antibody, a single chain fragment (scFv) derived from the 224-236-1 hybridoma (Westphal *et al*., 1997), to label the actin network by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AK423 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 224-236-1 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 were collected after 4 days; this antibody has a low production yield on our system (<10 mg/L).

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 224-236-1 supernatant (dilution 1:3 in PBS-BSA) and with the reformatted AK423 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

Despite the fact the AK423 antibody is produced at low yield, it labels the actin network, notably the cell cortex, pseudopods, endosomal compartments, and macropinocytic cups (Fig. 1). The staining with both antibodies appears almost indistinguishable (Fig. 1).

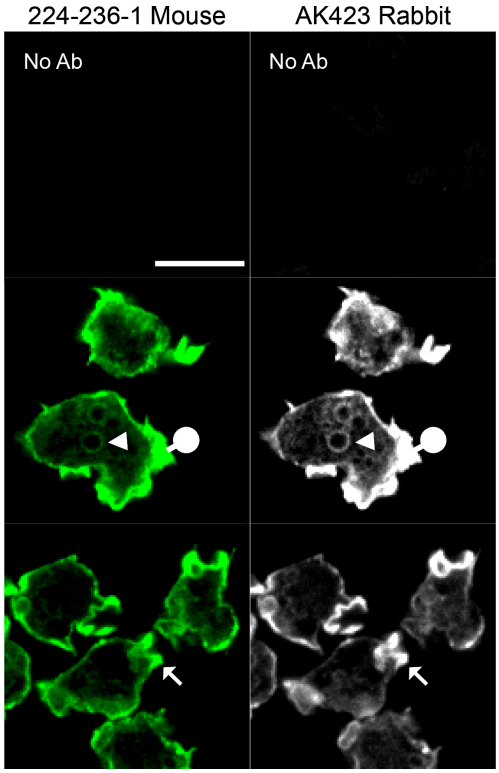

Fig. 1. The 224-236-1 hybridoma and the AK423 scFv antibody label the actin network in *Dictyostelium* cells, notably the cell cortex, pseudopods (pinheads), endosomal compartments (arrowheads) and macropinocytic cups (arrows). A double immunofluorescence staining with 224-236-1 and AK423 was performed. No labelling was seen when the primary antibodies were omitted (No Ab). Scale bar: 10 µm.

References

Westphal M, Jungbluth A, Heidecker M, *et al*. Microfilament dynamics during cell movement and chemotaxis monitored using a GFP-actin fusion protein. Curr Biol. 1997; 7(3):176-83. PMID:9276758

Conflict of interest

The authors declare no conflict of interest.