The AK426 antibody recognizes the Golgi apparatus in Dictyostelium cells by immunofluorescence

Wanessa Cristina Lima

Geneva Antibody Facility, Faculty of Medicine, University of Geneva, 1 rue Michel Servet, CH-1211, Geneva, Switzerland

Abstract

The AK426 antibody, derived from the 1/39 hybridoma, detects by immunofluorescence the Golgi apparatus from *Dictyostelium discoideum*.

Introduction

The 1/39 monoclonal antibody recognizes an unidentified antigen decorating the Golgi apparatus of *D. discoideum* (Gräf *et al*., 1999). Here we describe the ability of the AK426 antibody, a single chain fragment (scFv) derived from the 1/39 hybridoma, to label Golgi compartments by immunofluorescence.

Materials & Methods

Antibodies: ABCD_AK426 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 1/39 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 (~150 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 ºC 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 1/39 supernatant (dilution 1:3 in PBS-BSA) and with the reformatted AK426 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

Similarly to the original 1/39 hybridoma (Gräf *et al*., 1999; Merlot *et al*., 2003), the AK426 antibody labels the Golgi apparatus in a juxtanuclear position (Fig. 1).

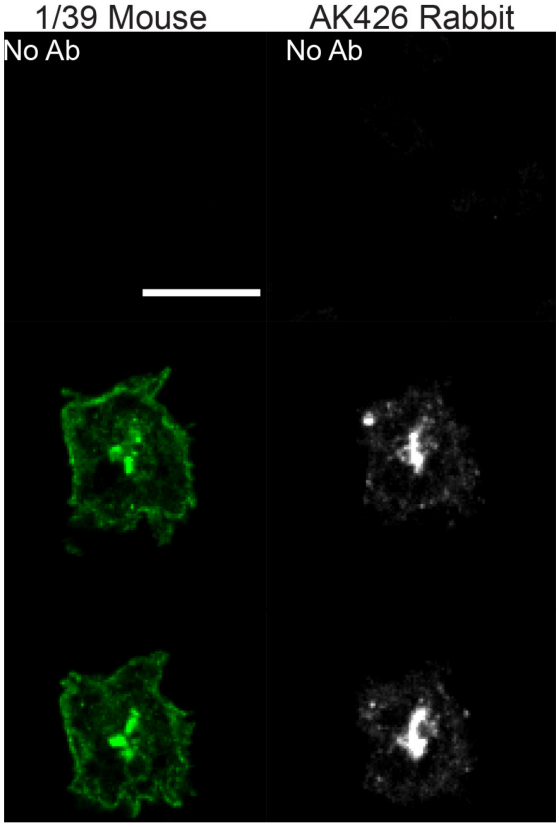

Fig. 1. The 1/39 hybridoma and the AK426 scFv antibody label the Golgi apparatus in *Dictyostelium* cells. A double immunofluorescence staining with 1/39 and AK426 was performed. No labelling was seen when the primary antibodies were omitted (No Ab). Scale bar: 10 μm.

References

Gräf R, Daunderer C, Schliwa M. Cell cycle-dependent localization of monoclonal antibodies raised against isolated Dictyostelium centrosomes. Biol Cell. 1999; 91(6):471-7. PMID: 10519007.

Merlot S, Meili R, Pagliarini DJ, Maehama T, Dixon JE, Firtel RA. A PTEN-related 5-phosphatidylinositol phosphatase localized in the Golgi. J Biol Chem. 2003; 278(41):39866-73. PMID: 12878591

Conflict of interest

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