The AK422 antibody recognizes Dictyostelium SctA protein by Western blot

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Abstract

The AK422 antibody, derived from the B4.2 hybridoma, detects by Western blot the full-length SctA protein from *Dictyostelium discoideum*.

Introduction

SctA (Secreted Protein A, Protein p17, DDB_G0278725, UniProt #O77257) is the most abundant protein in secreted pycnosomes (Sabra *et al*., 2016). Here we describe the ability of the AK422 antibody, a single chain fragment (scFv) derived from the B4.2 hybridoma, to detect the full-length *Dictyostelium* SctA protein by Western blot.

Materials & Methods

Antibodies: ABCD_AK422 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 B4.2 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 were collected after 4 days; this antibody has a low production yield on our system (<10 mg/L).

Antigen: *D. discoideum* DH1 cells, cultivated in HL5 medium, were used to detect the full-length protein.

Protocol: 10^6 and 10^7 cells were pelleted, 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; reduced samples were also boiled for 15 min at 95 °C). 10 µL of each sample ($5x10^4$ and $5x10^5$ cells) was migrated (150 V, 45 min) in a 4-20% acrylamide gel (Genscript, SurePAGE Bis-Tris, #M00655), 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 60 min 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 (PBS-Tween). The membranes were then incubated overnight at RT with either the B4.2 mouse monoclonal or the AK422 scFv antibody (diluted 1:5 or 1:2 in PBS-Tween, respectively). The membranes were then washed three times (15+15+10 min) and incubated for 1 hour with the horseradish peroxidase-coupled goat anti-mouse IgG (Biorad#170-6516, dilution 1:3000) or anti-rabbit IgG (Sigma #A8275, dilution 1:3000) and washed three times (15 min) in PBS-Tween. The signal was revealed by enhanced chemiluminescence (ECL) (Amersham Biosciences) using a PXi-4 gel imaging systems (Syngene).

Results

Despite the fact the AK422 antibody is produced at low yield, it specifically recognizes the SctA protein, detecting a single band around 15 kDa, similarly to the original B4.2 hybridoma (Fig. 1) (Sabra *et al*., 2016).

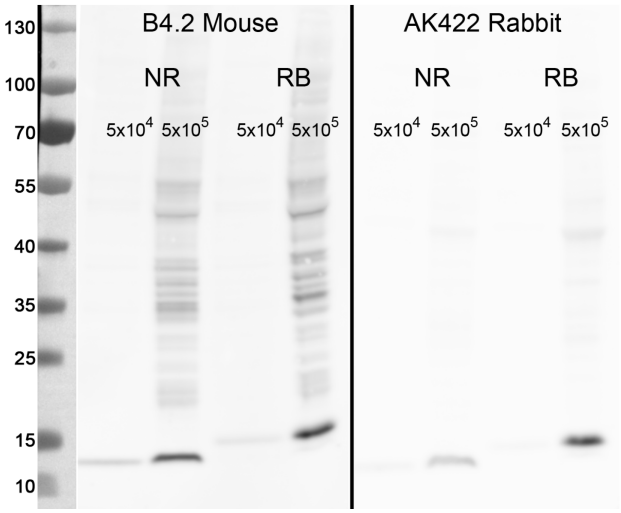

Fig. 1. The B4.2 hybridoma and the AK422 scFv antibodies specifically recognize the SctA protein (predicted molecular mass ~15 kDa). (NR: non-reducing conditions; RB: reducing and boiled conditions)

References

Sabra A, Leiba J, Mas L, *et al*. Pycnosomes: Condensed endosomal structures secreted by Dictyostelium amoebae. PLoS One. 2016; 11(5):e0154875. PMID: 27187592.

Conflict of interest

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

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