The AL626 antibody recognizes an ALFA-tagged recombinant protein by immunofluorescence

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Abstract

The AL626 antibody against the ALFA tag recognizes an ALFA-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells.

Introduction

The ALFA tag is a rationally designed epitope tag, forming a small (15 aa), stable α-helix, uncharged at physiological pH and resistant to chemical fixation. A nanobody (NbALFA) specifically recognizes this tag in native or fixed conditions, and is thus suitable for a large number of applications, including super-resolution microscopy, immunoprecipitation, western blotting, and electron microscopy (Götzke *et al.*, 2019). Here, we show that the AL626 recombinant antibody, derived from the NbALFA nanobody, detects an ALFA-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: The ABCD_AL626 antibody (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) was produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as a mini-antibody with the antigen-binding VHH fused to a mouse IgG2A Fc. The synthesized VHH sequence (GeneArt, Invitrogen) corresponds to the sequences of the variable region of the synthetic camelid antibody NbALFA (Götzke *et al.*, 2019). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (100 mg/L) was collected after 4 days.

Antigen: HeLa cells (growing in DMEM GlutaMAX™, Gibco #31966; supplemented with 8% Fetal Bovine Serum, Gibco #10270) cultured on glass coverslips (Menzel-Gläser, 22x22 mm) and transiently transfected 2 days before the experiment with an ALFA-tagged TAC protein (Uniprot #P01589), were used to detect the peptide tag. The ALFA epitope sequence used was `PSRLEEELRRRLTE` and it was present in the C-terminal cytosolic domain of the fusion protein. An antibody detecting the N-terminal extracellular domain of the TAC protein (AJ519, with rabbit IgG Fc; Arsimoles *et al.*, 2020) was used as a positive control. The ALFA-tagged TAC protein is expected to be mostly present at the cell surface.

Protocol: The whole procedure was carried out at room temperature. Transfected HeLa cells were rinsed once with PBS, fixed with PBS + 4% paraformaldehyde (w/v) (Applichem, #A3013) for 30 min, and blocked with PBS + 40 mM ammonium chloride (NH_4Cl) (Applichem, #A3661) for 5 min. Cells were then permeabilized in PBS + 0.2% saponin (w/v) (Sigma, #S7900) for 3 min, incubated in PBS + 0.2% (w/v) BSA (PBS-BSA) for 30 min, and then with the AL626 (final concentration 5 mg/L) and AJ519 antibodies (final concentration 2.5 mg/L in PBS-BSA) for 1 h. After 3 washes (10 min) with PBS-BSA, cells were incubated for 30 min in PBS-BSA with secondary goat anti-mouse IgG conjugated to AlexaFluor-647 and anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes, #A21235 and #A11034, respectively). After 3 washes (10 min) with PBS-BSA, cells were incubated during 10 min with DAPI (1:500, Molecular Probes, #D1306), washed twice with PBS-BSA and once with PBS, and 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

The AL626 antibody specifically detected a signal at the plasma membrane in cells transfected with the ALFA-tagged TAC protein (Fig. 1). The signal co-localized with the signal generated by the anti-TAC AJ519 antibody (Fig. 1, arrows); the specificity of the signal was further verified by the absence of both anti-TAC and anti-ALFA stainings in the few non-transfected cells (Fig. 1, arrowheads). No staining was observed when the primary antibody was omitted (Fig. 1, No Ab).

Conflict of interest

Pierre Cosson and Wanessa Cristina Lima are editors of the Antibody Reports journal.

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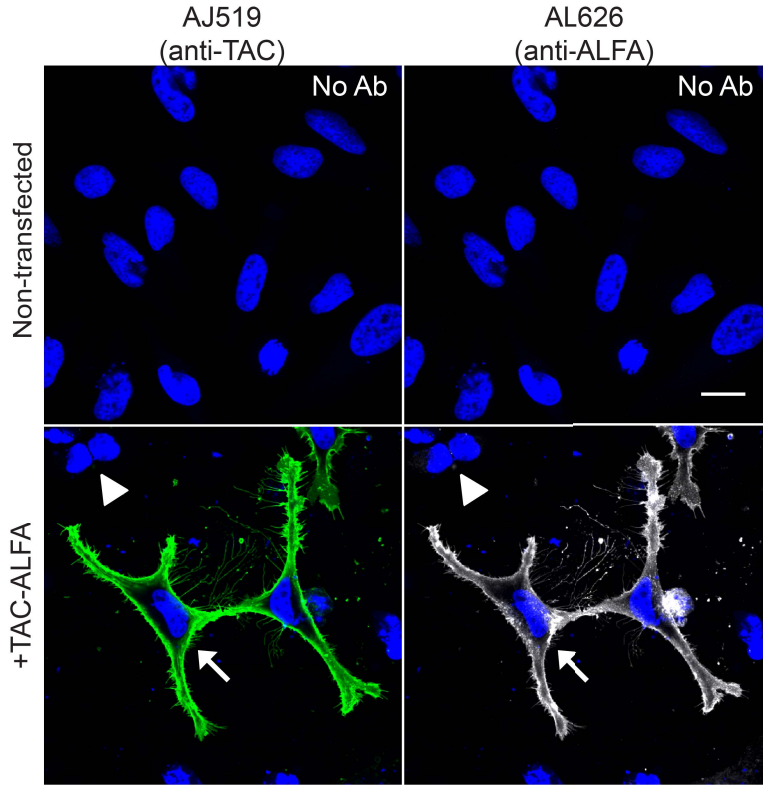

Fig. 1. AL626 labeled the plasma membrane of HeLa cells expressing the ALFA-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). Scale bar: 20 µm.