AF166 and AI179 antibodies recognize a Myc-tagged recombinant protein by immunofluorescence

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Abstract

AF166 and AI179 antibodies against the Myc tag recognize a Myc-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells; AF372, AF373 and AI831 do not.

Introduction

The Myc tag is a short hydrophilic peptide derived from the human proto-oncogene c-myc protein (Uniprot #P01106), extensively used for detection of tagged proteins using anti-Myc antibodies. The standard antibody developed to detect the Myc epitope (EQKLISEEDL) is the mouse monoclonal 9E10 clone (Evan *et al.*, 1985). Here, we show that the AF166 and AI179 recombinant antibodies, derived from slightly different 9E10 published sequences, detect a Myc-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: ABCD_AF166, ABCD_AF372, ABCD_AF373, ABCD_AI179, and ABCD_AI831 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a mouse IgG2A Fc. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the clones 9E10 (for AF166, AI179, and AI831, all three slightly different and published by different research groups; Schiweck *et al.*, 1997, Fuchs *et al.*, 1997, and Krauss *et al.*, 2008), 1A5 and 2C7 (for AF372 and AF373 respectively; Lee *et al.*, 2016) joined by a peptide linker $(GGGS)_3$. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (30 mg/L for AF372 and AF373) were collected after 4 days; AF166, AI179 and AI831 have a low production yield in this system (<5 mg/L).

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 a Myc-tagged TAC protein (Uniprot #P01589), were used to detect the peptide tag. The Myc epitope sequence used was `EQKLISEEDLL` 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 Myc-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 tested anti-Myc antibodies (final concentration 5 mg/L in PBS-BSA) for 1 h. When indicated, the AJ519 antibody was added to this incubation (final concentration 2.5 mg/L in PBS-BSA). 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 (1:300, Molecular Probes, #A21235) and, when AJ519 was used for co-localization, goat anti-rabbit IgG conjugated to AlexaFluor-488 (1:300, Molecular Probes #A11034). 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

Despite their low production yield, AF166 and AI179 antibodies specifically detected a signal at the plasma membrane in cells transfected with the Myc-tagged TAC protein (Fig. 1A). The signal co-localized with the signal generated by the anti-TAC AJ519 antibody (Fig. 1A, arrows); the specificity of the signal was further verified by the absence of both anti-TAC and anti-Myc stainings in the few non-transfected cells (Fig. 1A, arrowheads). No signal was seen with AF372 and AI831 antibodies; AF373 detected a weak signal, but the staining pattern does not correspond to the expected cell surface localization (Fig. 1B). No staining was observed when the primary antibody was omitted (Fig. 1A, No Ab).

Conflict of interest

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

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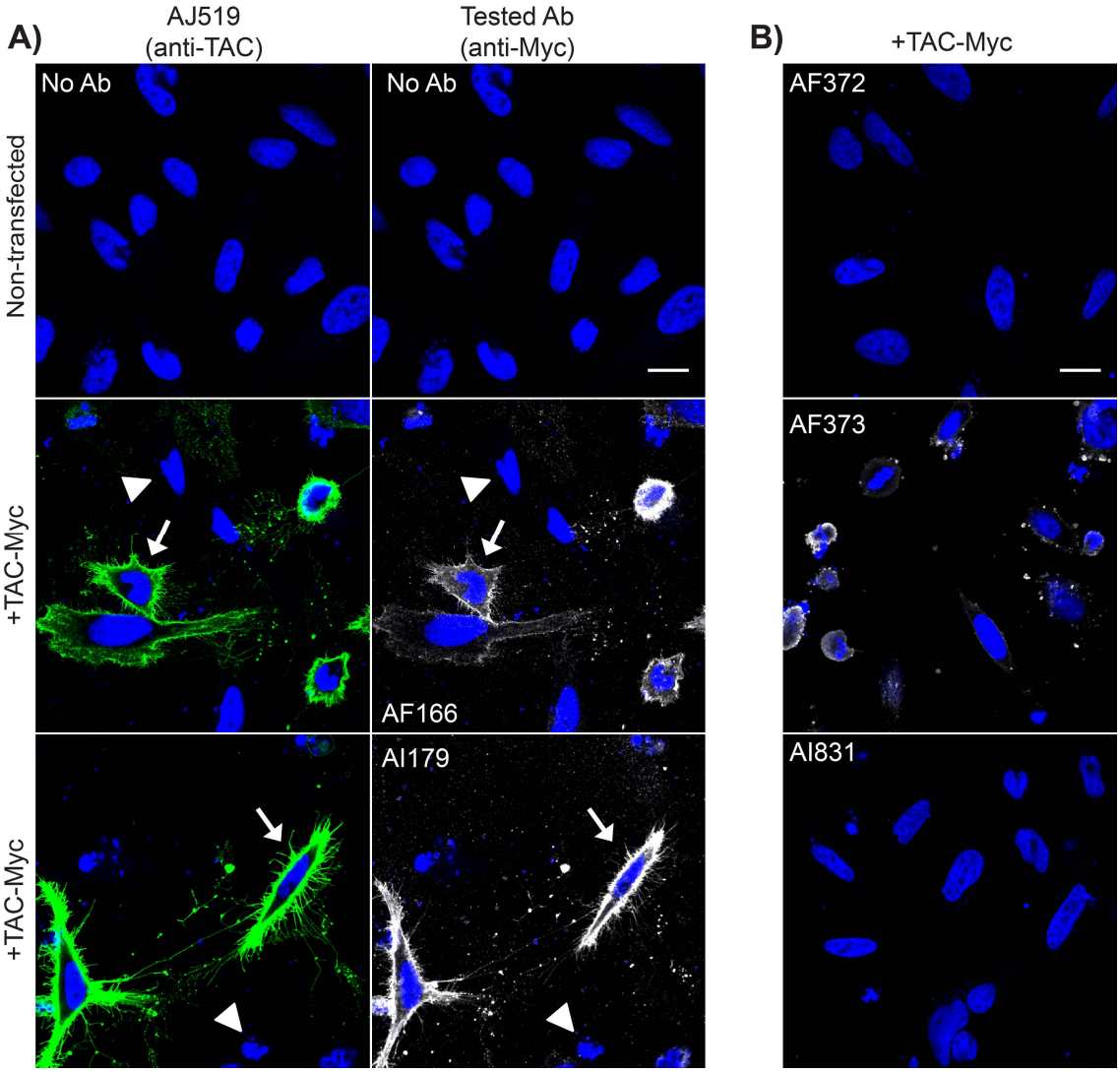

Fig. 1. (A) AF166 and AI179 labeled the plasma membrane of HeLa cells expressing the Myc-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). **(B)** No labelling was seen for AF372 and AI831; a faint labelling was seen for AF373, but not at the expected location. Scale bar: 20 µm.