

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

Wanessa Cristina Lima, Pierre Cosson

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

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 (NH₄Cl) (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 Mowiol (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

The authors declare no conflict of interest.

References

Arsimoles D, D'Esposito A, Gaspoz V, *et al.* The AJ519 antibody labels the human TAC/IL2RA protein by immunofluorescence. *Antibody Reports*, 2020, 3:e118. doi:10.24450/journals/abrep.2020.e118

Evan GI, Lewis GK, Ramsay G, Bishop JM. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol Cell Biol*. 1985; 5(12):3610-6. PMID:3915782

Fuchs P, Breitling F, Little M, Dübel S. Primary structure and functional scFv antibody expression of an antibody against the human protooncogen c-myc. *Hybridoma*. 1997; 16(3):227-33. PMID: 9219032.

Krauss N, Wessner H, Welfle K, *et al.* The structure of the anti-c-myc antibody 9E10 Fab fragment/epitope peptide

complex reveals a novel binding mode dominated by the heavy chain hypervariable loops. *Proteins*. 2008; 73(3):552-65. PMID: 18473392.

Lee SS, Park JE, Lim JC, Yoo SH. c-Myc specific antibodies. Republic of Korea; KR1020160133368, 2016.

Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Res*. 2020; 48(D1):D261-D264. PMID:31410491

Schiweck W, Buxbaum B, Schätzlein C, Neiss HG, Skerra A. Sequence analysis and bacterial production of the anti-c-myc antibody 9E10: the V(H) domain has an extended CDR-H3 and exhibits unusual solubility. *FEBS Lett*. 1997; 414(1):33-8. PMID:9305727

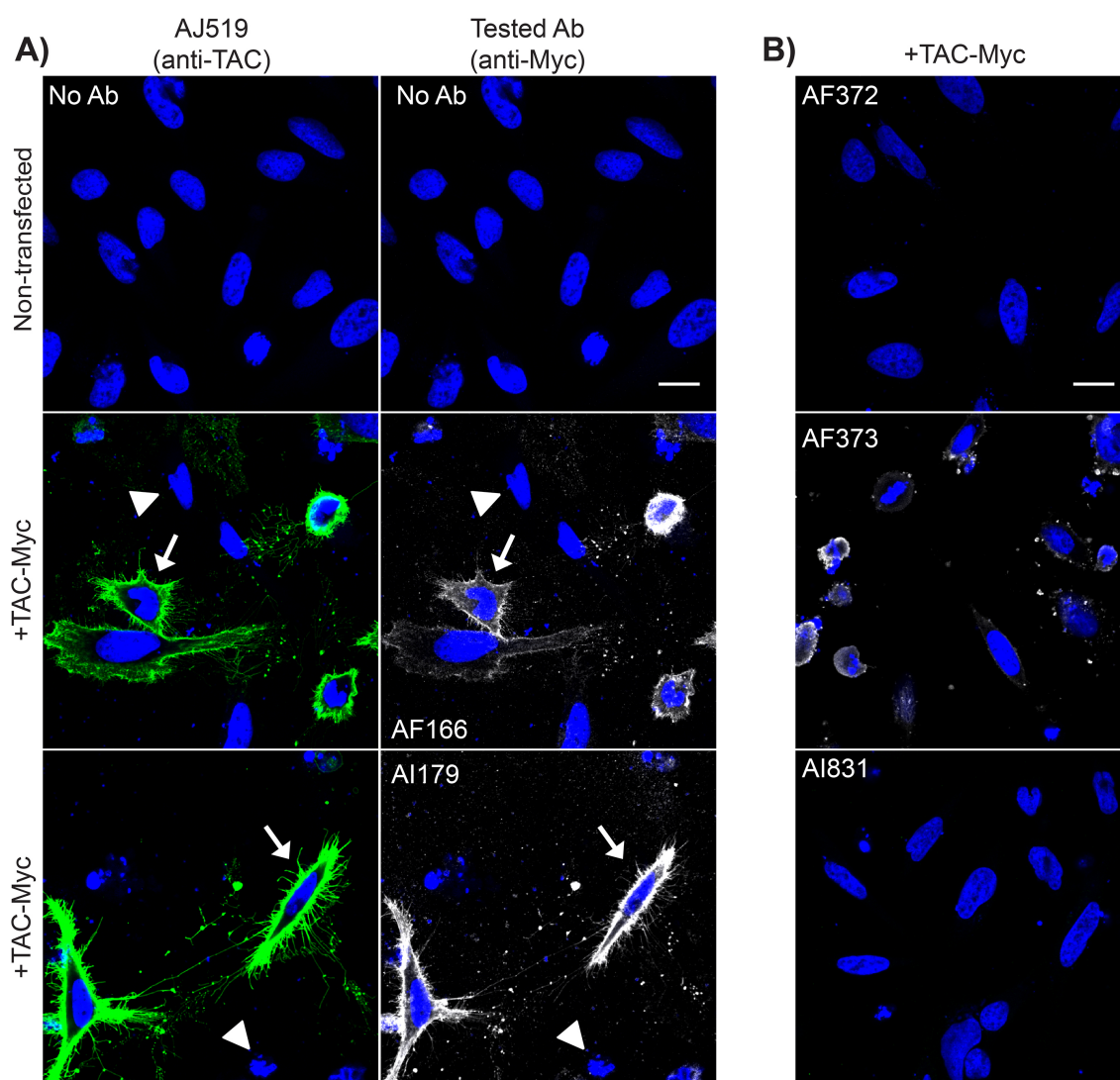


Fig. 1. (A) AF166 and AI179 labeled the plasma membrane of HeLa cells expressing the Myc-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. No labelling was seen when the primary 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 μ m.