The AI215 antibody recognizes an EPEA-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

The AI215 antibody against the EPEA tag recognizes an EPEA-tagged human TAC protein by immunofluorescence in paraformaldehyde-fixed HeLa cells.

Introduction

The EPEA tag (also called C tag) is an extremely short epitope derived from the human α-Synuclein protein (Uniprot #P37840), used for detection and purification of tagged proteins with the NbSyn2 antibody (De Genst *at al.*, 2010). Here, we show that the AI215 recombinant antibody, derived from the NbSyn2 nanobody, detects an EPEA-tagged human TAC protein by immunofluorescence in HeLa cells.

Materials & Methods

Antibodies: The ABCD_AI215 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 NbSyn2 (Pardon *et al.*, 2013). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatant (90 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 EPEA-tagged TAC protein (Uniprot #P01589), were used to detect the peptide tag. The EPEA epitope sequence used was `GGEPEA` 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 EPEA-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 AI215 (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 AI215 antibody specifically detected a signal at the plasma membrane in cells transfected with the EPEA-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-EPEA 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

The authors declare no conflict of interest.

Geneva University Library Open Access Publications
https://oap.unige.ch/journals/abrep | ISSN 2624-8557

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:10.22450/journals/abrep.2020.e118

De Genst EJ, Guilliams T, Wellens J, *et al*. Structure and properties of a complex of α-synuclein and a single-domain camelid antibody. J Mol Biol. 2010; 402(2):326-43. PMID:20620148

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

Pardon E, Steyaert J, Wyns L. Epitope tag for affinity-based applications. USA; US20130115635, 2013.

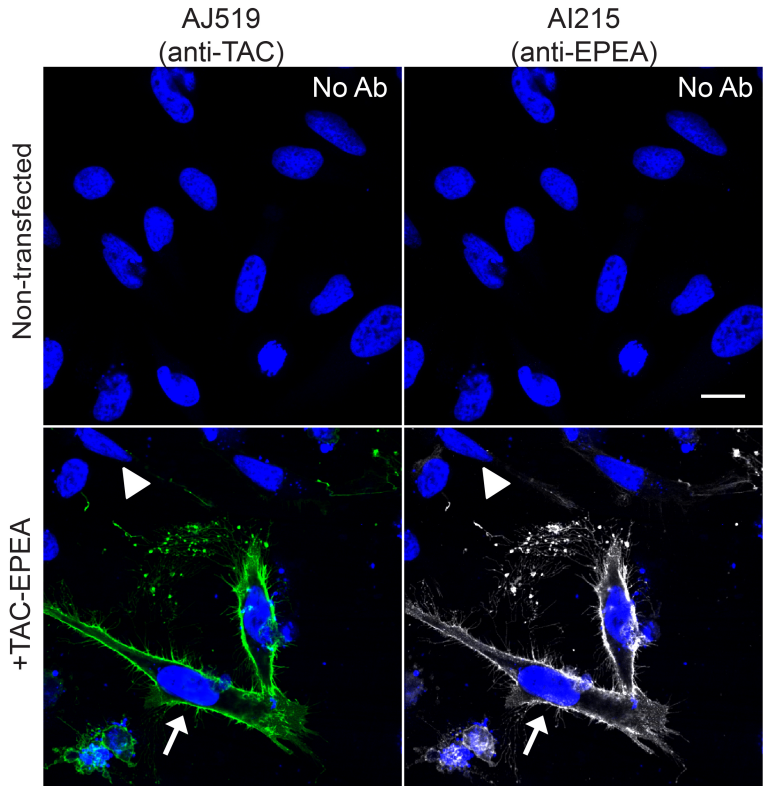

Fig. 1. AI215 labeled the plasma membrane of HeLa cells expressing the EPEA-tagged TAC protein (in white); the signal co-localized (arrows) with the signal generated by the anti-TAC AJ519 antibody (in green); in blue, nuclei were stained with DAPI. No labelling was seen when the primary antibody was omitted, or in non-transfected cells (arrowheads). Scale bar: 20 µm.