The ABCD_AI993 antibody does not label the human GAPDH protein by western blot

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Abstract

The ABCD_AI993 antibody does not detect the human GAPDH protein by western blot in HEK cell lysates.

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; UniProt #P04406) is an enzyme involved in glycolysis which converts glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate. It plays a key metabolic role and is involved in cellular functions such as apoptosis and gene expression regulation (Nicholls *et al*., 2012). The ABCD_AI993 (AI993) antibody was characterized as an anti-GAPDH scFv sequence isolated from B cells in multiple sclerosis plaques (Kolln *et al*., 2006). This sequence is referenced under the accession ID ABCD_AI993 in the ABCD database (ABCD nomenclature, http://web.expasy.org/abcd/). In our experiments, the AI993 antibody did not detect the endogenous GAPDH protein by western blot in HEK293 cells.

Materials & Methods

Antibodies: The AI993 antibody was produced by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/). It was produced as a mini-antibody with the antigen binding scFv portion fused to a rabbit IgG Fc. The synthesized scFv sequence (GeneArt, Invitrogen) corresponds to the sequence of the variable regions of the clone 3260-9-1 (Kolln *et al*., 2006) joined by a peptide linker $(GGGGS)_4$. The antibody was produced in HEK293 suspension cells growing in HEK TF medium (Xell #861-0001, Sartorius) supplemented with 0.1% Pluronic F68 (Sigma #P1300). Cells were transiently transfected using FectoPro® transfection reagent (Polyplus #101000014) with the vector coding for the scFv-Fc of AI993 and supernatant was collected after 3 days. As a positive control, a commercial monoclonal mouse anti-GAPDH antibody was used (Proteintech #60004-1-Ig).

Protocol: $5x10^5$ HEK293 cells were pelleted, washed once in PBS and lysed in PBS containing 0.5% (v/v) Triton X-100. A centrifugation step (5 min at 12'000 g) allowed to discard the pelleted nuclei and to recover the supernatants containing GAPDH which were mixed with reducing or non-reducing 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). Each sample was separated by electrophoresis on an acrylamide gel 4-20% (SurePAGE Bis-Tris, Genscript #M00655) at 200 V for 30 min. Transfer was performed onto a nitrocellulose membrane using a dry transfer system for 10 min (iBlot gel transfer device, Invitrogen #IB1001EU). Membranes were blocked 2 h in PBS containing 0.1% (v/v) Tween20 and 7% (w/v) milk (PBS-Tween-milk) and washed three times for 5 min in PBS + 0.1% (v/v) Tween20 (PBS-Tween). The membranes were then incubated with AI993 antibody at a concentration of 5 mg/L or with the positive control diluted 1:20'000 in PBS-Tween-milk for 20 min at room temperature and washed three times for 5 min with PBS-Tween. The membranes were then incubated for 20 min with either the horseradish peroxidase-coupled goat anti-rabbit IgG (Sigma-Aldrich #A8275, dilution 1:3000 in PBS-Tween-milk) or the horseradish peroxidase-coupled goat anti-mouse IgG (Sigma-Aldrich #A11029, dilution 1:3000 in PBS-Tween-milk). After 2 washes for 5 min in PBS-Tween, the signal was revealed by enhanced chemiluminescence (ECL) (Millipore) using a PXi-4 gel imaging system (Syngene).

Results and discussion

The AI993 antibody was used to detect endogenous GAPDH in HEK cell lysates by western blot. One band was observed at approximately 40 kDa with a commercial antibody used as a positive control (CTR+), under both reducing (R) and non-reducing (NR) conditions (Fig. 1). This is slightly higher than the expected theoretical molecular weight of 36 kDa. This discrepancy may be due to post-translational modifications. No signal was detected with the AI993 antibody under either reducing or non-reducing conditions. This antibody thus failed to detect the GAPDH protein by western blot, contrary to its original description (Kolln *et al*., 2006). This lack of reactivity in our hands could be due to the fact that the antibody was formatted in this study as a mini-antibody with the scFv fused to Fc fragments. Alternatively, variations in experimental conditions, may explain why our results differ from those published previously (Kolln *et al*., 2006). This negative result does not exclude the possibility that

ABCD_AI993 may detect the GAPDH protein in other experimental setups and assays. This possibility remains to be tested.

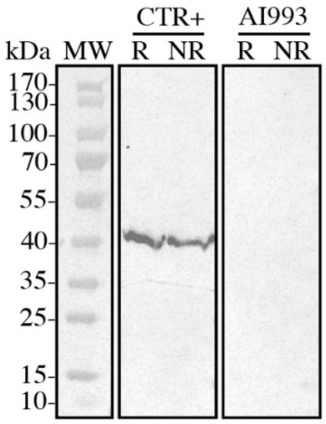

Fig. 1. Detection of GAPDH by western blot in HEK cell lysates. One band at approximatively 40 kDa was detected in reducing conditions (R) and in non-reducing conditions (NR) conditions with a commercial anti-GAPDH antibody used as a positive control (CTR+). No band was observed with the antibody AI993.

References

Nicholls, C., Li, H., & Liu, J. P. (2012). GAPDH: a common enzyme with uncommon functions. *Clinical and experimental pharmacology & physiology*, *39*(8), 674–679.PMID: 21895736.

Kolln, J., Ren, H. M., Da, R. R., Zhang, Y., Spillner, E., Olek, M., Hermanowicz, N., Hilgenberg, L. G., Smith, M. A., van den Noort, S., & Qin, Y. (2006). Triosephosphate isomerase- and glyceraldehyde-3-phosphate dehydrogenase-reactive autoantibodies in the cerebrospinal fluid of patients with multiple sclerosis. *Journal of immunology (Baltimore, Md. : 1950)*, *177*(8), 5652– 5658. https://doi.org/10.4049/jimmunol.177.8.5652.

Conflict of interest

Tania Jauslin is an editor of the Antibody Reports journal.