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AG424 and AG427 antibodies recognize the human TrkB protein by western blot

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Abstract

The recombinant antibodies AG424 and AG427 detect the human TrkB protein in the human neural progenitor cell line ReNcell® VM and in human brain by western blot.

Introduction

TrkB (Tropomyosin-related kinase B; UniProt Q16620) is the receptor for BDNF (brain-derived neurotrophic factor) and NTF4 (neurotrophin-4). Upon ligand-binding, it activates different effector cascades responsible for regulation of neuronal differentiation, growth and survival (Traub *et al.*, 2017). Here, we describe the ability of two recombinant TrkB-agonistic antibodies, AG424 and AG427, to detect endogenous TrkB by western blot.

Materials & Methods

Antibodies: ABCD_AG424 and ABCD_AG427 (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/antibodies/) as mini-antibodies with the antigen-binding scFv fused to the Fc region of a mouse IgG2A. The synthesized scFv sequences (GeneArt, Invitrogen) correspond to the sequences of the variable regions of the anti-TrkB clones A10/Ab20 (for AG424; Blaustein, 2010; Traub *et al.*, 2017) and C2 (for AG427; Lin *et al.*, 2010), joined by a peptide linker $(GGGGS)_3$. HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium, Gibco 12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (20 and 40 mg/L, respectively) were collected after 4 days.

Antigen: The human neural progenitor cell line ReNcell® VM (Merck Millipore SCC008) was used. A knockout of the *NTRK2* gene was performed by CRISPR-Cas9 technology using specific plasmids from Santa Cruz (sc-400142 and sc-400142-HDR). A specific clone was identified and sequenced to confirm the cassette insertion in the *NTRK2* gene. WT and KO ReNcell® VM cells were cultivated on Matrigel coated dishes (Corning 354277), in Neurobasal medium (Gibco 21103049) supplemented with B-27 (Gibco 17504044), sodium pyruvate (Gibco 11360039), non-essential amino-acids, (Gibco 11140035), Glutamax (Gibco 35050061) and basic fibroblast growth factor (bFGF) 20 ng/mL (Brunschwig APOBITP2079), epidermal growth factor (EGF) 20 ng/mL (Cell Guidance System GFH26) and Penicillin-Streptomycin 100 U (Gibco 15140122). Differentiation of ReNcell® VM cells was initiated by adding medium without bFGF and EGF for 5 days. Human cortex was used as positive control. Recombinant human BDNF (50 ng/mL) (Gibco PHC7074) was added to Neurobasal medium when specified. After 5 minutes, medium was removed and washed with PBS. Cells were collected in lysis buffer with a cell scraper.

Protocol: Cell pellets were extracted in lysis buffer (Tris 100 mM, NP40 2%, SDS 0.2%, NaCl 0.3 M, Na deoxycholate 1%, protease inhibitor cocktail without EDTA), sonicated and centrifuged. Proteins recovered in the supernatant were dosed with Pierce BCA Protein Assay (ThermoFisher 23227). 40 µg of each sample was prepared in loading buffer (Tri-HCl pH 6.8 175 mM, glycerol 15%, SDS 5%, DTT 33 mM, bromophenol blue), heated (100 °C, 7 min) and migrated (100 V, 30 min) in a 8% acrylamide gel (Tris base pH 8.8 0.375 M, acrylamide 4K 8% [Applichem A1672], SDS 0.1%, ammonium persulfate 0.05%, TEMED 1:1000 [Sigma T9281]) in running buffer (Tris base 25 mM, glycine 192 mM, SDS 0.1%). Samples were transferred (80 V, 90 min) to a PVDF 0.45 µm membrane (Millipore IPVH00010) in transfer buffer (Tris base 25 mM, glycine 192 mM, pH adjusted between 8.1 and 8.4, methanol 20%, SDS 0.01%). Membranes were colored with FastGreen (0.01% in methanol 50% and acetic acid 12.5%), rinsed with TBS buffer (Tris-HCl 15.76 g/l, Tris base 12.14 g/l, NaCl 8.8 g/l) + Tween 20 (0.05% v/v) before proceeding to immunoblotting.

Anti-TrkB staining: Membranes were blocked 1h in TBS buffer containing Tween 20 (0.05% v/v) (TBST) and milk (5% v/v), and incubated with AG424 (1:25; 0.8 µg/mL in blocking buffer) or AG427 (1:50; 0.8 µg/mL in blocking buffer) overnight at 4 °C. Membranes were washed 3 times with TBST, and incubated with horseradish peroxidase-coupled anti-mouse IgG (1:5000, Biorad 170-6516). Membranes were washed 5 times in TBST. The signal was revealed by enhanced chemiluminescence ECL (Advansta WesternBright Sirius K-12043-D10) using a Fusion solo (Witec AG).

Anti-Actin staining: Membranes were cut under 77 kDa, blocked 1h in TBST containing milk (5% v/v), and incubated with anti-actin alpha clone 4 (Merck MAB1501) (1:100000 in blocking buffer) overnight at 4 °C. Membranes were washed 3 times with TBST, and incubated with horseradish peroxidase-coupled anti-mouse IgG (1:5000, Biorad 170-6516). Membranes were washed 5 times in TBST. The signal was revealed by enhanced chemiluminescence ECL (Advansta WesternBright Quantum #K-12042-D10) using a Fusion solo (Witec AG).

Results

Antibody AG424 (Fig. 1A) specifically recognized three bands in WT ReNcell® VM and in human cortex: a ~145 kDa band corresponding to full-length TrkB, a higher molecular weight band, likely a TrkB dimer, and a smaller band at ~90 kDa corresponding to a truncated form, which lacks the intracellular catalytic kinase domain (Klein *et al.*, 1990). These three bands were absent from *TRKB*-KO cells, confirming their specificity. Antibody AG427 recognized the ~145 kDa full length TrkB band as well as the dimerization product. However, AG427 appeared to be less specific, as additional bands were visible and the smaller band was hardly detected (Fig. 1B). FastGreen protein staining and actin labelling were used as loading controls. Stimulation of cells with BDNF enhanced the formation of the TrkB dimer.

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Conflict of interest

The authors declare no conflict of interest.

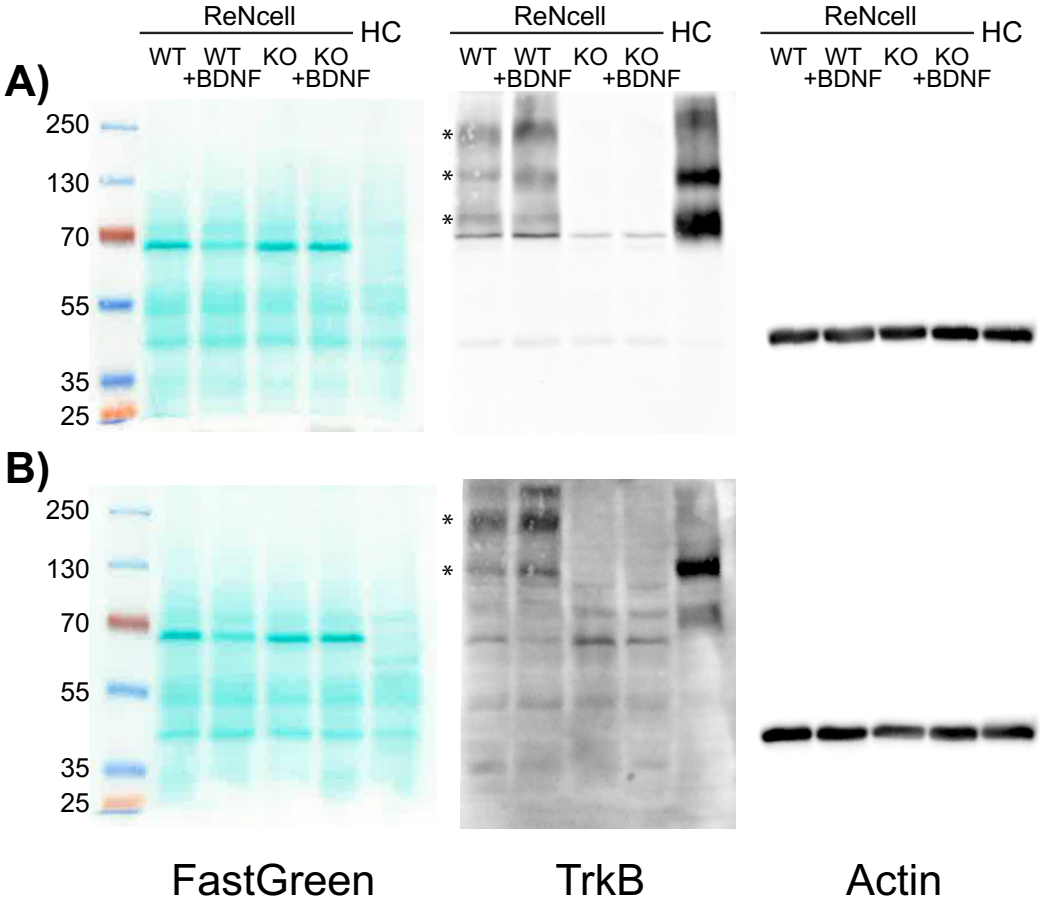

Fig. 1. Specific binding of AG424 (A) and AG427 (B) antibodies to endogenous TrkB in WT ReNcell® VM and human cortex (HC) (positions indicated by asterisks). TRKB-KO cells ('KO') were used as a negative control. Protein staining with FastGreen or detection of actin levels (with an anti-actin antibody) were used as loading controls. "+BDNF": cells stimulated with BDNF.