ABCD_RB905 antibody recognizes an extracellular epitope of the endogenous human latrophilin-2 protein by immunofluorescence

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Abstract

The recombinant antibodies ABCD_RB904, ABCD_RB905, ABCD_RB906, ABCD_RB907, ABCD_RB908 and ABCD_RB909 detect by ELISA a synthetic peptide located within the extracellular domain of the human latrophilin-2 (LPHN2). ABCD_RB905 recognizes the endogenous LPHN2 by immunofluorescence.

Introduction

Latrophilin-2 (LPHN2, UniProt # O95490) is an adhesion G-protein-coupled receptor best known for its role in neuronal development and synapse formation (Anderson 2017). Emerging evidence indicate that it is widely distributed across tissues and versatile in various pathological contexts (Lee 2022). LPHN2 structure typically comprises a C-terminal signaling domain and an extended N-terminal extracellular domain. This latter domain plays a pivotal role in mediating complex interactions with cell surface molecules and extracellular matrix components, thus influencing its functional roles beyond traditional GPCR signaling. There are few commercial antibodies against LPHN2 and they are not well characterized. Indeed, developing antibodies to identify LPHN2 is a big challenge due to the protein's complex structure and its potential of changing conformations depending on the protein's active state. Here we describe the ability of six recombinant antibodies, ABCD_RB904-909, to detect by ELISA a synthetic biotinylated peptide from the human LPHN2 protein. This peptide is part of the extracellular olfactomedin-like domain. One of these antibodies is further characterized for its capacity to detect endogenous LPHN2 in the Calu-3 airway epithelial cell line.

Materials & Methods

Antibodies: The antibodies ABCD_RB904, ABCD_RB905, ABCD_RB906, ABCD_RB907 and ABCD_RB908, ABCD_RB909 (ABCD nomenclature, http://web.expasy.org/abcd/, referred to collectively as RB904–909) were generated by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies/). Briefly, a semi-synthetic phage display library (Novimmune) was panned against the biotinylated peptide corresponding to amino acids 335 to 357 of the human LPHN2 protein (P1, sequence:TGKNSIDYIYNTRLNRGEYVDVP). After three rounds of panning, selected phage vectors were isolated using a plasmid preparation kit (Qiagen), and the scFv inserts were subcloned into custom-made expression vectors and sequenced. The selected antibodies were expressed as mini-antibodies, consisting of the antigen-binding scFv domain fused to a mouse IgG1 Fc region. HEK293 suspension cells, cultured in HEK TF medium (Sartorius #861-0001) supplemented with 0.1% Pluronic F68 (Sigma #P1300), were transiently transfected with the vectors encoding the scFv-Fc constructs. Supernatants containing the mini-antibodies were harvested three days post-transfection. Estimated production yields were approximately between 50–120 mg/L.

Antigen: The same peptide used for phage selection served as the antigen for ELISA detection. For negative control, an alternative N-biotinylated peptide derived from the cytoplasmic region of human LPHN2 protein (P2, residues 1293–1331, sequence:GSSSEDDAIVADASSLMHSDNPGLELHHK ELEAPLIPQR) was utilized.

ELISA: The whole procedure was carried out at room temperature. Biotinylated peptides at saturating concentration (10 pmol/well) were immobilized on streptavidin-coated ELISA plates (Pierce #15124) for 30 min. Each well was rinsed three times with 100 µl of washing buffer (PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween20), then incubated for 1 hour with 50 µl of RB antibody-containing supernatant diluted in washing buffer (Fig. 1). After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (Bio-Rad #170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 3 rinses, Tetramethylbenzidine (TMB) substrate (Sigma #T5569) was added (50 µl per well). The reaction was stopped by the addition of 25 µl of 2 M H_2SO_4. The absorbance (OD) was measured at 450 nm, and the absorbance at 570 nm was subtracted.

Immunofluorescence: To detect the ability of RB905 to recognize endogenous LPHN2 in a human cell line, Calu-3 epithelial cells, cultured in Minimum Essential Medium GlutaMAX supplemented with 1% non-essential amino acids, 1% HEPES, 1% sodium pyruvate, 10% heat-

inactivated Fetal Bovine Serum (ThermoFisher #10270-106) and Penicillin/Streptomycin/Fungizone (Bioconcept #4-02F00-H) at 37°C in humidified 5% CO2 atmosphere, were fixed for 30 min with 4% paraformaldehyde (Sigma #158127). Cells were permeabilized for 15 min with 0.2% Triton X-100 (Sigma #T8787) buffer before being blocked for an hour with the PBS 1% BSA saturation buffer. Cells were then incubated with RB905 primary antibody at 1/10 dilution for 2 hours at room temperature, washed and then incubated with secondary goat anti-Mouse IgG antibody conjugated with AlexaFluor-647 (ThermoFisher #A21236 for 1 hour in the dark. Cells were then washed and mounted with Vectashield (Vector Laboratories #H-1000). Images were captured using a stellaris 63x oil objective. Specificity of the antibody was tested in a Calu-3 cell line knockdown for LPHN2 by CRISPR-Cas9 using Lentiviral particles for sgRNA and Cas9 nuclease (GeneCopoeia).

RNA-sequencing: Total RNA was extracted from Calu-3 cells and sequenced by the *i*GE3 Genomic Platform at the Faculty of Medicine, University of Geneva. The raw data files can be accessed to NCBI Gene Expression Omnibus (GEO) with the accession number GSE133495.

Results

The chosen peptide sequence is predicted to be in a beta sheet structure and is not homologous to any known protein, with the exception of Latrophilin-3 (LPHN3), which shares 53% homology.

Antibodies RB904-RB909 bound in a concentration-dependent manner to the extracellular LPHN2 P1 peptide against which they were raised, but not to the negative control P2 peptide (Fig. 1). We next studied the ability of RB905 to bind the full-length endogenous protein by immunofluorescence.

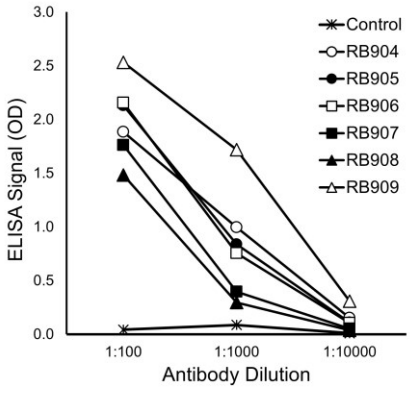

Fig. 1. Specific binding of RB antibodies to the target LPHN2 peptide, as detected by ELISA. 'Control' indicates the binding of RB904 to the negative control peptide (all other control curves were superimposed).

RB905 detected LPHN2 in Calu-3 epithelial cell line (Fig. 2A). Labeling strongly localized at cell-cell contacts, and it was significantly decreased in cells with LPHN2 knockdown (Fig.2B). No signal was found in the absence of the primary antibody (Fig.2C). Noteworthy, Calu-3 cells express relatively low levels of LPHN3 compared to LPHN2 (Fig. 2D). This emphasizes the signal's specificity for LPHN2 in Calu-3. However, the possibility of cross-reactivity with LPHN3 should be carefully considered in other cell types.

Future studies should examine the capacity of other antibodies to bind endogenous LPHN2.

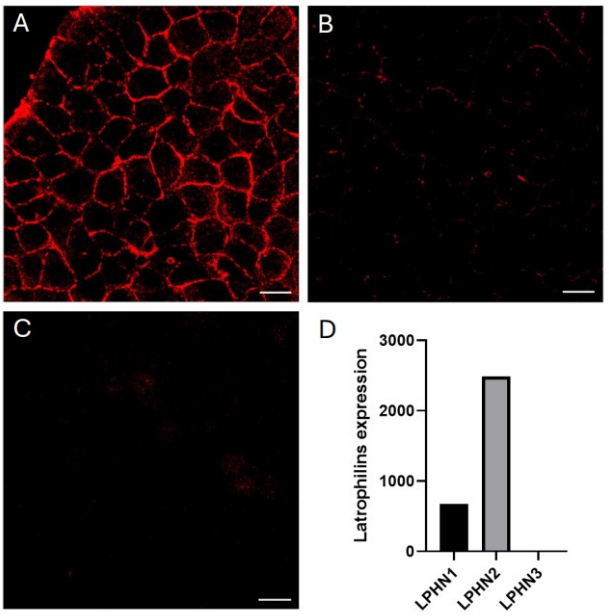

Fig. 2. RB905 recognizes LPHN2 (red) in Calu-3 cells (A). A significant decrease of the labeling was observed in Calu-3 with LPHN2 knockdown (B). No signal was detected when the first antibody was omitted (C). Latrophilins genes' expression profile in Calu-3 cells by RNA sequencing (D). Scale bars: 20 µm

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Conflict of interest

The authors declare no conflict of interest.