ABCD_RB881-RB886 antibodies recognize a human lipocalin2 peptide by ELISA

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Abstract

The recombinant antibodies ABCD_RB881, ABCD_RB882, ABCD_RB883, ABCD_RB884, ABCD_RB885 and ABCD_RB886 detect by ELISA a synthetic biotinylated peptide from the human lipocalin2 protein.

Introduction

Lipocalin2 (LCN2/NGAL, UniProt #P80188) is a multifunctional protein member of the adipokine family that binds bacterial siderophores to sequester iron, playing a key role in innate immunity. LCN2 interacts with its receptor (NGALR) to regulate inflammation and has been linked to neurodegenerative diseases through its stabilization of MMP-9, a matrix metalloproteinase involved in blood-brain barrier integrity (Chandrasekaran *et al*., 2024). LCN2 is also used as a biomarker of kidney injury and dysfunction (Devarajan, 2010).
Here we describe the ability of six recombinant antibodies (ABCD_RB881-RB886) to detect by ELISA a synthetic biotinylated peptide from the human LCN2 protein.

Materials & Methods

Antibodies: ABCD_RB881, ABCD_RB882, ABCD_RB883, ABCD_RB884, ABCD_RB885 and ABCD_RB886 antibodies (ABCD nomenclature, http://web.expasy.org/abcd/, referred to collectively as RB881-886) were discovered by the Geneva Antibody Facility (http://unige.ch/medecine/antibodies). Briefly, a synthetic VHH phage display library (in-house) was panned against a LCN2 biotinylated peptide (see antigen section). After three rounds of panning, selected phage vectors were isolated using a plasmid preparation kit (Qiagen), and the VHH inserts were subcloned into custom-made expression vectors and sequenced. The selected antibodies were produced as mini-antibodies with the antigen-binding VHH portion fused to a human IgG1 Fc. HEK293 suspension cells (growing in HEK TF medium, Xell #861-0001, Sartorius, supplemented with 0.1% Pluronic F68, Sigma #P1300) were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (40-130 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to amino acid 110 to 128 of the human LCN2 protein sequence (GEFTLGNIKSYPGLTSYLV). The same peptide was used in the ELISA assay. As a negative control, an irrelevant N-biotinylated peptide (AEFSMDDFEDTFDS NATISTKDLFEGSDRLPLNQSINTTIQNL) from *Dictyostelium discoideum* stat5 protein (UniProt #O00910) was used.

Protocol: 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-human IgG (BioRad #1721050, 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.

Results and Conclusion

The ability of the six recombinant antibodies RB881–886 to bind the human LCN2 peptide was evaluated by ELISA. An irrelevant peptide from *D. discoideum* was used as a negative control. As shown in figure 1, antibodies RB881-886 bound in a concentration-dependent manner to the LCN2 peptide against which they were raised, but not to the negative control peptide (Fig. 1). Antibody RB883, produced at a relatively high concentration (100 mg/L), displayed a weaker binding signal in the ELISA assay, suggesting a lower apparent affinity for the target peptide compared with the other antibodies. Although these antibodies recognize specifically the LCN2 peptide by ELISA, their ability to bind the full-length protein should be determined in future experiments.

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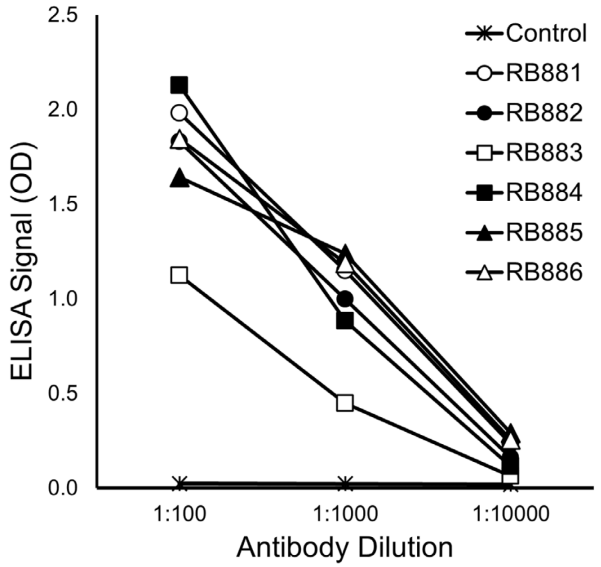

Fig. 1. Specific binding of the tested antibodies to the target LCN2 peptide, as detected by ELISA. 'Control' indicates the binding of RB881 to the negative control peptide from *D. discoideum* stat5 protein (all other control curves were superimposed).

References

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Conflict of interest

The authors declare no conflict of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.