RB706, RB707, RB708, RB709, RB710 and RB711 antibodies recognize a human ROR2 peptide by ELISA

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Abstract

The recombinant antibodies RB706, RB707, RB708, RB709, RB710 and RB711 detect by ELISA a synthetic peptide from the ROR2 protein.

Introduction

Tyrosine-protein kinase-like orphan receptor ROR2, also known as neurotrophic tyrosine kinase, receptor-related 2 (NTRKR2) is a tyrosine kinase transmembrane receptor which is overexpressed in the cartilage during development (DeChiara *et al*., 2000). Here we describe the ability of six recombinant antibodies (RB706, RB707, RB708, RB709, RB710 and RB711) to detect by ELISA a synthetic biotinylated peptide from the ROR2 protein.

Materials & Methods

Antibodies: ABCD_RB706, ABCD_RB707, ABCD_708, ABCD_709, ABCD_710, and ABCD_RB711 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were discovered by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) and produced as mini-antibodies with the antigen-binding scFv portion fused to a human IgG1 Fc (HRB706, HRB707, HRB708, HRB709, HRB710 and HRB7011). HEK293 suspension cells (growing in HEK TF medium, Xell#861-0001, supplemented with 0.1% Pluronic F68, Sigma#P1300) were transiently transfected with the vector coding for the scFv-Fc of each antibody. Supernatants (~5 s- 100 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to 14 residues (THSPNHNFQDDYHE). from ROR2 protein sequence (UniProt # Q01974)**.** As a negative control, an irrelevant N-biotinylated peptide (RTVYMESLHMQGEI) from ROR1 protein (UniProt #Q01973) 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 HRB 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

Antibodies RB706, RB707, RB708, RB709, RB710 and RB7011 bound in a concentration-dependent manner to the ROR2 peptide against which they were raised, but not to the negative control peptide (Fig. 1).

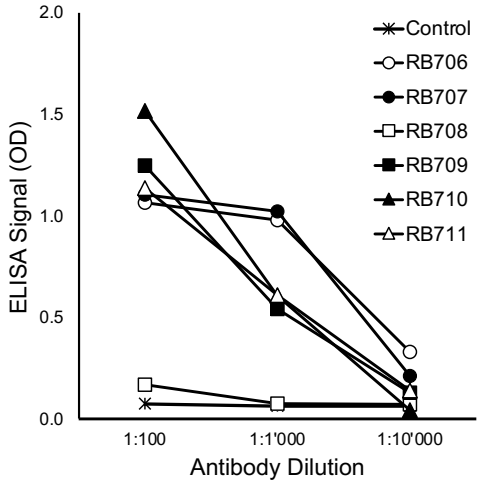

Fig. 1. Specific binding of HRB antibodies to the target ROR2 peptide, as detected by ELISA. 'Control' indicates the binding of HRB706 to the negative control peptide (all other control curves were superimposed).

References

DeChiara, T. M., R. B. Kimble, W. T. Poueymirou, J. Rojas, P. Masiakowski, D. M. Valenzuela, and G. D. Yancopoulos. 2000. "Ror2, Encoding a Receptor-like Tyrosine Kinase, Is Required for Cartilage and Growth Plate Development." Nature Genetics 24 (3): 271–74. doi.org/10.1038/73488.

Conflict of interest

The authors declare no conflict of interest.