RB608, RB609, RB610, RB611 and RB612 antibodies recognize murine pancreatic polypeptide by ELISA

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Abstract

Newly generated recombinant antibodies RB608, RB609, RB610, RB611 and RB612 detect by ELISA the peptidic hormone termed pancreatic polypeptide (PPY).

Introduction

Pancreatic polypeptide (PPY, UniProt P10601) is a hormone secreted by pancreatic gamma cells (also known as PP cells) of the endocrine pancreas. It regulates pancreatic and gastrointestinal functions (Yonekura *et al.*, 1988). Until the recent development of an efficient mouse monoclonal antibody, no reliable anti-PPY antibodies were commercially available (Hara *et al.*, 2019). Here, we describe the ability of five recombinant antibodies (RB608, RB609, RB610, RB611 and RB612) to recognize mouse pancreatic polypeptide by ELISA.

Materials & Methods

Antibodies: ABCD_RB608, ABCD_RB609, ABCD_RB610, ABCD_RB611 and ABCD_RB612 antibodies (ABCD nomenclature, https://web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (https://www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding VHH portion fused to a mouse IgG2A Fc. HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco 12338) were transiently transfected with the vector coding for the VHH-Fc of each antibody. Supernatants (~50-100 mg/L) were collected after 4 days.

Antigen: The antibodies were raised against a N-biotinylated synthetic peptide corresponding to the processed, mature form (residues 30-65) of the murine pancreatic polypeptide (`APLEPMYPGDYATPEQMAQYETQLRRYINTLTRPRY`). As control, an irrelevant peptide (`HFERPRGPRLGYSIPSRSGASGLDKRDYV`, from human CLDN9, UniProt O95484) 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. After rinsing 3 times (100 µl washing buffer), wells were incubated with horseradish peroxidase-coupled goat anti-mouse IgG (BioRad 170-6516, dilution 1:1000, 50 µl per well) for 30 min. After 5 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.

Results

Antibodies RB608, RB609, RB610, RB611 and RB612 bound in a concentration-dependent manner to a peptide comprising residues 30-65 of murine PPY, but not to an irrelevant control peptide (Fig. 1).

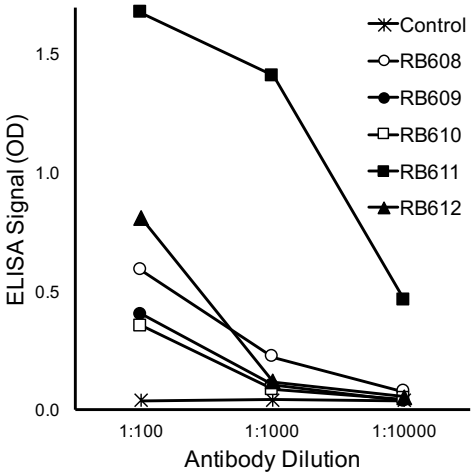

Fig. 1. RB608, RB609, RB610, RB611 and RB612 antibodies bind specifically to murine pancreatic polypeptide, but not to an irrelevant control peptide, as detected by ELISA. Control is shown only for RB608; all other background curves are superimposed.

References

Hara A, Nakagawa Y, Nakao K, *et al*. Development of monoclonal mouse antibodies that specifically recognize pancreatic polypeptide. Endocr J. 2019; 66(5):459-468. PMID: 30842364.

Yonekura H, Nata K, Watanabe T, Kurashina Y, Yamamoto H, Okamoto H. Mosaic evolution of prepropancreatic polypeptide. II. Structural conservation and divergence in pancreatic polypeptide gene. J Biol Chem. 1988; 263(6):2990-7. PMID: 3343236.

Conflict of interest

The authors declare no conflict of interest.