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AE804, AE806, AE999, AF017, AF041 and AF131 antibodies label mouse insulin-secreting beta cells by immunohistochemistry

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Abstract

The recombinant antibodies AE804, AE806, AE999, AF017, AF041 and AF131 detect by immunohistochemistry the insulin-secreting beta cells in mouse pancreatic islets. The most efficient staining was obtained with AE999.

Introduction

Pancreatic islet cells are highly differentiated and can be characterized by the distinctive hormones they produce: insulin for beta cells, glucagon for alpha cells, somatostatin for delta cells and pancreatic polypeptide for PP cells (Baskin, 2015). Here, we describe the ability of six recombinants antibodies (AE804, AE806, AE999, AF017, AF041 and AF131) to detect pancreatic insulin-secreting beta-cells by immunohistochemistry.

Materials & Methods

Antibodies: ABCD_AE804, ABCD_AE806, ABCD_AE999, ABCD_AF017, ABCD_AF041 and ABCD_AF131 antibodies (ABCD nomenclature, web.expasy.org/abcd/; Lima *et al.*, 2020) were produced by the Geneva Antibody Facility (www.unige.ch/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc. The synthesized scFv sequences (GeneArt, Invitrogen) for AE804, AE806, AE999, AF017, AF041 and AF131 correspond to the sequence of the variable regions of the CG7C7 (or mAb 126), AE9D6 (or mAb 125), HB125, mAb49, 193020 and M16 clones, respectively (Ewulonu *et al.*, 1990; Lake *et al*., 1994; Ikematsu *et al*., 1994; Pléau *et al*., 1993; Oyu and Thomas, 1997), joined by a peptide linker $(GGGGS)_3$. HEK293 suspension cells (growing in FreeStyleTM 293 Expression Medium, Gibco #12338) were transiently transfected with the vector coding for the scFv-Fc. Supernatants (for AE804, AE806, AE999 and AF131, ~50 mg/L) were collected after 4 days; production of AF017 and AF041 was undetectable in this system, indicating a low production yield (<5 mg/L).

Antigen: AE804, AE806, AE999 and AF131 were originally raised against human insulin (Uniprot #P01308) in mice (Ewulonu *et al.*, 1990; Lake *et al*., 1994; Oyu and Thomas, 1997); AF017 and AF041 were originally identified as autoantibodies from either human patients with diabetes mellitus (Ikematsu *et al*., 1994) or from diabetic mice (Uniprot #P01326; Pléau *et al*., 1993) respectively.

Protocol: Mouse pancreas was surgically removed, fixed in PBS + 4% paraformaldehyde during 2 h at RT. Pancreas sections were performed at the Histology Core Facility of the Geneva medical school, Switzerland. First, pancreas samples were dehydrated in an automatic tissue processing machine (Histokinette, Leica) in successive baths of ethanol at growing concentrations (70% twice 2 hs, 90% once 1 h, 95% once 1 h, 100% three times 1 h), followed by incubation in a solvent bath (three times 1 h; HistoSav, Biosystems #39-0591-05) and in bathes of liquid paraffin (three times 1 h; Leica #39601006). Samples were then embedded in liquid paraffin, cooled until solidification, and sectioned into 5 µm thick slices. Sections were deparaffinized and rehydrated with a series of alcohol solutions of decreasing concentrations (5 min in each solution, 100%, 95%, 70% and 50%) and kept in phosphate buffer saline (PBS) 30 min. Antigen retrieval was performed on deparaffined sections immersed in citrate 10 mM in PBS, pH 6 and microwave heated (once 7 min at 650 W and twice 5 min at 350 W). After blocking the endogenous peroxidase for 7 min with Dako blocking agent (Dako #SM801), slides were washed three times for 5 min in PBS, and incubated for 30 min at RT with the primary antibody (5 µg/ml for AE804, AE806, AE999, AF131, or less than 5 µg/ml for AF017 and AF041) in antibody dilution buffer (DAKO #DM830). After three washes (5 min) in PBS, slides were incubated 30 min at RT with horseradish peroxidase-coupled anti-rabbit IgG (DAKO #SM802). Slides were then washed three times for 5 min in PBS, and incubated with AEC (3-amino-9-ethylcarbazole) chromogenic substrate (BioGenex #HK129-5K) until signal formation (5 min). A counter-staining with hemalum was done for 2 min. Slides were mounted in aqueous mounting medium (Aquatex, Sigma-Aldricht #108562) and scanned with an Olympus VS120 microscope.

Results

Beta cells of pancreatic islets were successfully stained by AE804, AE806, AE999 and AF131 antibodies directed against insulin, showing a strong intracellular signal (Fig. 1), strongest with AE999. A lower signal was observed with AF017 and AF041 antibodies; this is most probably due to the fact that these antibodies are poorly produced, and thus used at a concentration lower than other antibodies. No staining was observed in pancreas sections in which the primary antibody was omitted (Fig. 1, negative control).

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Conflict of interest

The authors declare no conflict of interest.

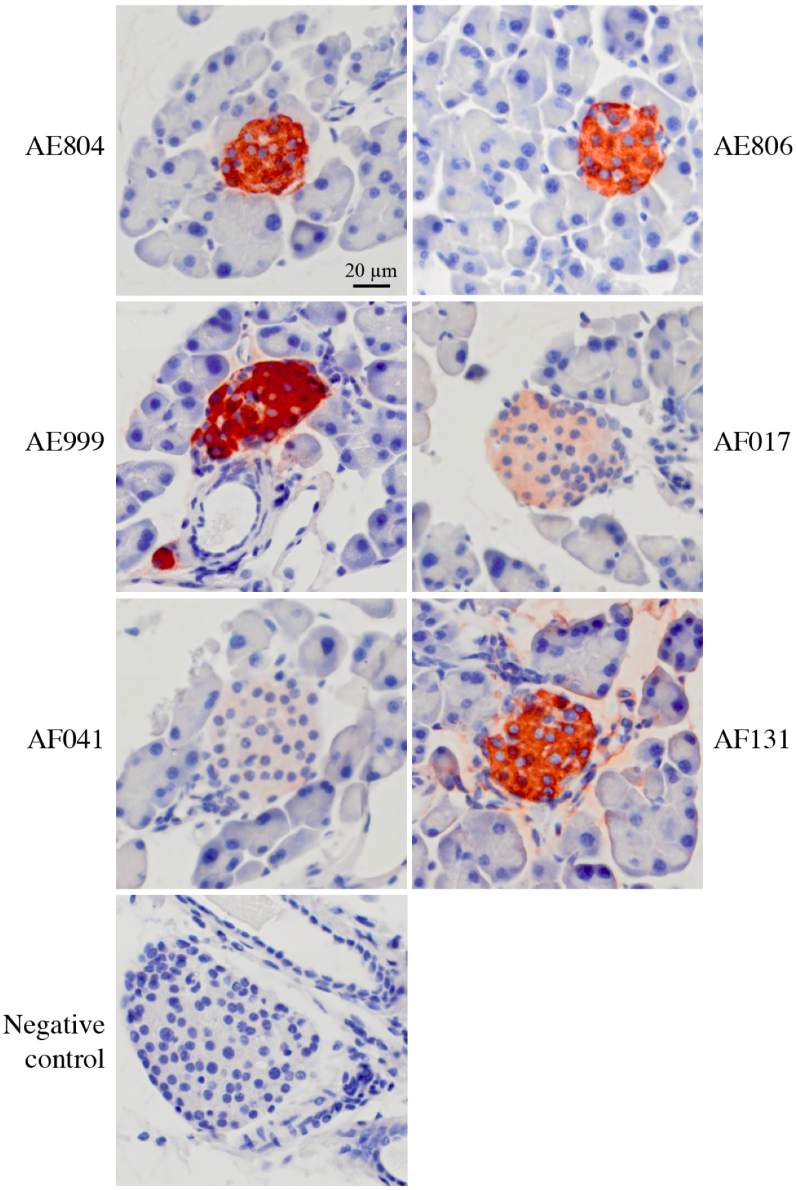

Fig. 1. AE804, AE806, AE999, AF017, AF041 and AF131 antibodies bind specifically to pancreatic insulin-secreting beta cells, as detected by immunohistochemistry. No staining was observed when the primary antibody was omitted (negative control). Scale bar: 20 µm.