TA010 and TA011 antibodies label mouse glucagon-secreting alpha cells by immunohistochemistry

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Abstract

The recombinant antibodies TA010 and TA011 detect by immunohistochemistry the glucagon-secreting alpha cells in mouse pancreatic islets.

Introduction

Pancreatic islets typically consist of four types of secretory endocrine cells, namely, insulin-containing beta cells, glucagon-containing alpha cells, somatostatin-containing gamma cells, and pancreatic polypeptide-producing (PP) cells (Baskin, 2015). It is generally accepted that these cells are not randomly distributed into islets. In most rodents, beta cells are at the center of the islets whereas alpha cells are located at the periphery (Orci *et al*., 1975). Here we describe the ability of two recombinant antibodies (TA010 and TA011) to detect mouse pancreatic glucagon-secreting alpha cells by immunohistochemistry.

Materials & Methods

Antibodies: ABCD_TA010 and ABCD_TA011 antibodies (ABCD nomenclature, www.expasy.org/abcd/) were synthetized and produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/) as mini-antibodies with the antigen-binding scFv fused to a rabbit IgG Fc. 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 (80 mg/L and 100 mg/L for TA010 and TA011 respectively) were collected after 4 days. The previously characterized antibody ABCD_AK247 (<5 mg/L) was used as a positive control (Okamoto and Gromada, 2016; Oppliger *et al*., 2021)

Antigen: All antibodies were originally raised against the human glucagon (Uniprot #P01275).

Protocol: Mouse pancreas was surgically removed, fixed in PBS + 4% paraformaldehyde during 2 h at room temperature (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 and gradually concentrated ethanol baths (70% twice 2 h, 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 baths 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) for 30 min. Antigen retrieval was performed on deparaffinized sections immersed in citrate 10 mM in PBS, pH 6 and microwave heated (7 min once at 650 W and 5 min twice at 350 W). Sections were washed for 10 min in PBS and then incubated for 30 min at RT with the primary antibodies (1/100 for TA010/TA011 and 1/10 for AK247) in antibody dilution buffer (DAKO #S2022). After three washes (5 min) in PBS, slides were incubated 20 min at RT with biotin-coupled anti-rabbit IgG (DAKO #K5001). Slides were then washed twice for 5 min in PBS and incubated for 20 min at RT with horseradish peroxidase-coupled streptavidin (DAKO #K5001). After two washes (5 min) in PBS, slides were incubated with DAB chromogenic substrate (DAKO #K5001) until signal formation. Slides were then washed two times for 5 min in PBS and counter-stained with hemalum for 2 min. Sections were mounted in aqueous mounting medium (Aquatex, Sigma- Aldricht #108562) and scanned with Grundium Ocus 40 microscope scanner.

Results

The anti-glucagon antibodies TA010 and TA011 generated a strong intracellular signal in cells located at the periphery of the islets (Fig. 1). The pattern of the signal observed with TA010 and TA011 is comparable to that obtained with the previously characterized AK247 antibody but with a higher level of background. It strongly suggests that these antibodies recognize specifically the alpha cells. The higher level of background might be explained by the higher amount of TA010/TA011 antibodies (>0.8 mg/L) used for the staining and/or by a lower affinity of these two antibodies compared to AK247 (<0.5 mg/L). No staining was observed in pancreas sections when the primary antibody was omitted (Fig. 1, negative control).

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Conflict of interest

Tania Jauslin is an associate-editor of the journal Antibody Reports.

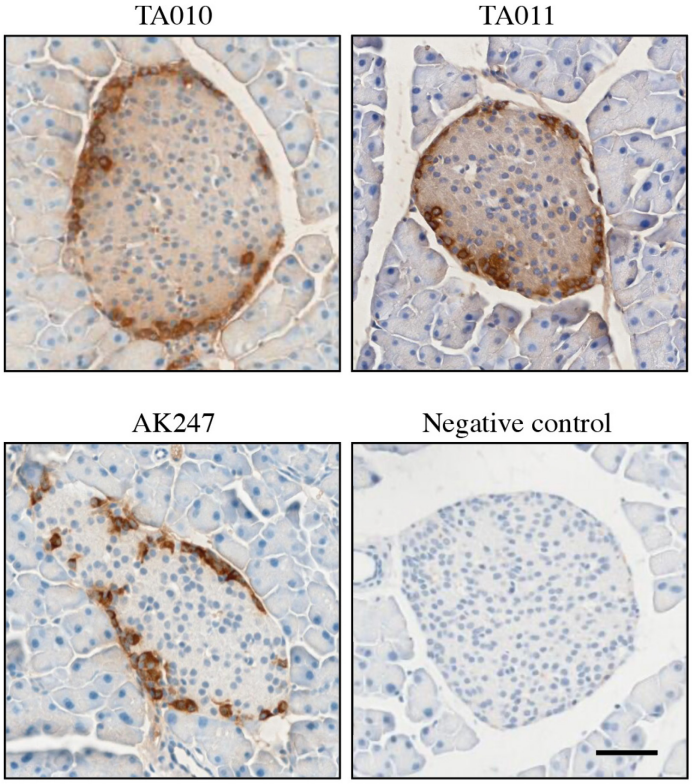

Fig. 1. TA010 and TA011 antibodies bind specifically to pancreatic glucagon-secreting alpha cells, as detected by immunohistochemistry. The observed signal is similar to the signal obtained with the previously characterized AK247 antibody, with a higher level of background signal. No staining was observed when the primary antibody was omitted (negative control). Scale bar: 50 µm**.**