RB459 and RB462 antibodies recognize mouse Pannexin1 protein by immunofluorescent staining

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Abstract

The recombinant antibodies RB459 and RB462 recognize mouse Pannexin1 (Panx1) by immunofluorescent staining of a mouse cardiac endothelial cell line, a murine macrophage cell line and cryosections of mouse tissue.

Introduction

Panx1 (Pannexin1; Uniprot #Q9JIP4) is a ubiquitously expressed protein that forms channels in the cell membrane, allowing the exchange of small molecules (<1.5 kDa), metabolites and ions between the intra and extracellular compartments. As such, Panx1 contributes to signaling between the cell and its environment in both physiological and pathological conditions (Molica *et al.*, 2018). Panx1 consists of four trans-membrane domains, two extracellular loops, and the intracellular loop, amino-terminus and carboxy-terminus are all located in the cytoplasm (Esseltine and Laird, 2016).

Here, we describe the ability of two recombinant antibodies, RB459 and RB462, to recognize a specific sequence in the C-terminal part of murine Panx1 by immunofluorescent staining on cells and tissue cryosections.

Materials & Methods

Antibodies: ABCD_RB459 and ABCD_RB462 antibodies (ABCD nomenclature, web.expasy.org/abcd/) were produced by the Geneva Antibody Facility (www.unige.ch/medecine/antibodies/; Blanc *et al*., 2014) as mini-antibodies with the antigen-binding scFv fused to a human IgG1 Fc (HRB459 and HRB462). HEK293 suspension cells (growing in FreeStyle™ 293 Expression Medium, Gibco #12338) were transiently transfected with the vectors coding for each scFv-Fc. Supernatants (~20-100 mg/L) were collected after 5 days.

Antigen: The antibodies were raised against a N-terminal biotinylated synthetic peptide corresponding to the residues 414 to 425 of Panx1 protein (EKNSRQRLLNPS) (Locovei *et al.*, 2006; Molica *et al.*, 2015).

Protocol: HRB462 was used to recognize Panx1 in immunofluorescent staining on a mouse cardiac endothelial cell line (MCECs) and a macrophage cell line (RAW 264.7) cultured on coverslips (22x22 cm, Huberlab, #10.0360.88). The procedure was carried out at room temperature (unless indicated differently). Cells were rinsed with phosphate-buffered saline (PBS, Gibco, #10010023) before a 15 min fixation with 4% (w/v) paraformaldehyde (PFA, Merck, #104005), permeabilized with 0.3% TritonX-100 (Axonlab, #A1388,0500) in PBS for 15 min and incubated with 0.5 M NH_4Cl (Sigma Aldrich, #213330) in PBS for 15 min. After each incubation step, samples were washed 3 times for 5 min with PBS. Blocking was performed with 2% bovine serum albumin (BSA, Applichem, #A1391.0100) in PBS for 30 min. Cells were stained overnight with HRB462 (1/250 in PBS) at 4 ^{o}C. After washing, samples were incubated for 60 min in BSA-PBS containing secondary goat anti-human IgG conjugated to AlexaFluor-647 (1/2000, Invitrogen, #A21445) and washed again. In order to visualize nuclei, a 10 min incubation with DAPI (Invitrogen, #D1306, 1/20'000 in PBS) was performed. Finally, samples were mounted on slides (ThermoFisher Scientific, #11800AMN7) using Vectashield (Vectorlabs, #H-1000).

To detect Panx1 in murine tissues frozen in optimal cutting temperature (OCT) compound (VNR, #361603E), we stained 5 µm cryosections of hearts and atherosclerotic lesions in carotid arteries from wild-type (WT) or Panx1 knock-out ($Panx1^{-/-}$) mice with HRB459 antibody. Before tissue collection, a perfusion was performed with 0.9 % NaCl (Sigma, #71376) via the left ventricle, while the right atrium was incised, in order to remove the blood from the vasculature. Hearts and carotid arteries were collected, rinsed with PBS, frozen in OCT and sectioned. The staining was carried out as above-mentioned with minor modifications in order to improve the quality of the fluorescent signal and to reduce the background signal. The modifications were: (1) sections were fixed in 1% PFA in PBS for 15 min; (2) sections were blocked with 10% normal goat serum (NGS, Vectorlabs, #S-1000) in PBS for 15 min; (3) HRB459 antibody was incubated at a dilution range of 1/25 to 1/100 in NGS/PBS overnight at 4 ^{o}C; (4) secondary goat anti-human IgG antibody conjugated with AlexaFluor-488 (Invitrogen, #A11013) was prepared in NGS/PBS (1:2000); and (5) an additional tissue counterstaining during 10 s with 0.003 % Evans blue (Sigma, #E2129) was included.

All images were captured using a Zeiss Axio Imager Z1 with a EC Plan-Neofluar 40x/1.3 Oil (420462.9900) or Plan Apochromat 63x/1.4 Oil (420782.9900) objectives.

Results

HRB462 specifically detects Panx1 (in red) in MCECs (Fig. 1A) and RAW 264.7 cells (Fig. 1C). No staining was observed in absence of the primary antibody (Fig. 1B and D). HRB459 was used to detect Panx1 in cryosections of murine tissues. As shown in Fig. 1E, the antibody recognizes Panx1 (in green) in the endothelium of coronary vessels (arrowhead) and in the endocardium (arrow) of WT mice. In addition, Panx1 (in green) was also detected in the cardiac microvasculature of WT mice (Fig. 1G). Finally, specific staining was also observed in atherosclerotic lesions present in carotid arteries of WT mice (Fig. 1I). No signal was detected at corresponding locations in tissues extracted from Panx1$^{-/-}$ mice (Fig. 1F, H, J).

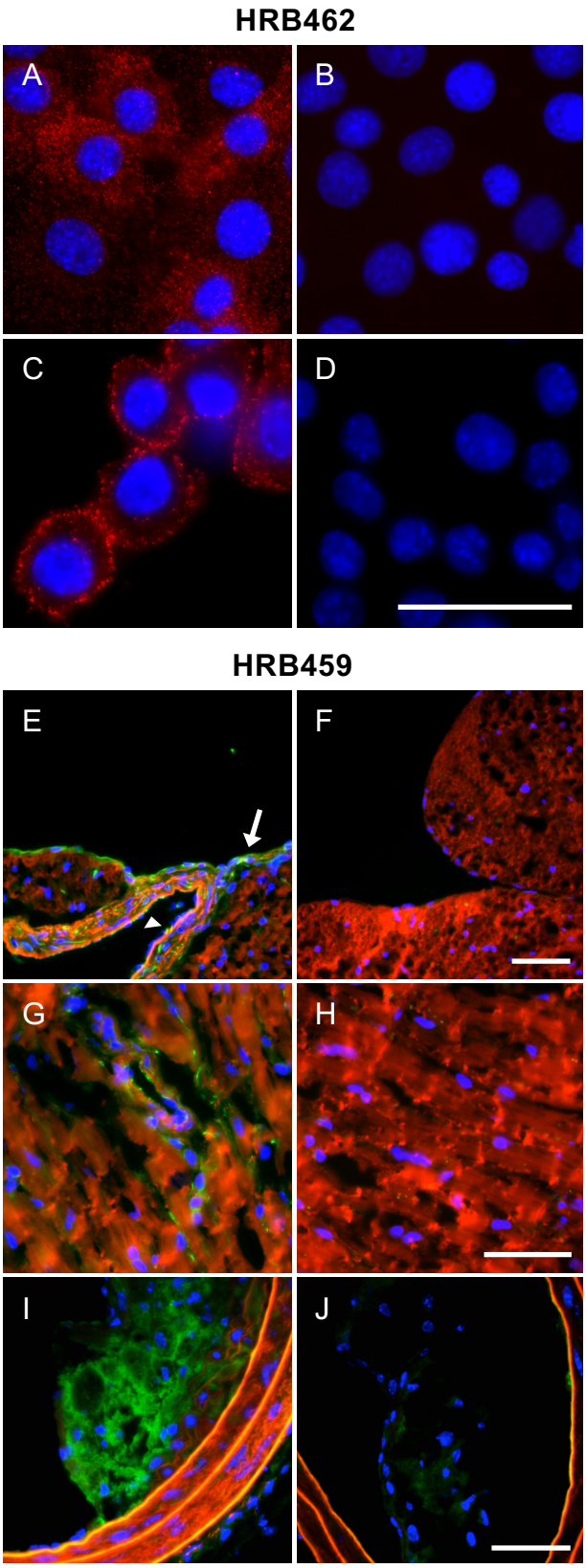

Fig. 1. (A-D) HRB462 recognizes Panx1 (red) in MCECs (A) and RAW 264.7 (C) cell lines. No signal was detected when the first antibody was omitted (B and D). DAPI (blue). (E-J) HRB459 targets Panx1 (green) in the endothelium of coronary vessels, in cardiac microvasculature and in atherosclerotic lesions in carotid arteries (E, G, I, respectively). No labeling was observed in tissues extracted from Panx1$^{-/-}$ mice (F, H and J). DAPI (blue), Evans blue (red). Scale bars: 50 µm.

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Conflict of interest

The authors declare no conflict of interest.