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Short Communication

Anti-Bovine Podoplanin Monoclonal Antibody PMab-44 Detects Goat Podoplanin in Immunohistochemistry

Shinji Yamada,[1] Mika K. Kaneko,[1] Yoshikazu Furusawa,[1–3] Shunsuke Itai,[1,4] Masato Sano,[1] Takuro Nakamura,[1] Miyuki Yanaka,[1] Saori Handa,[1] Kayo Hisamatsu,[1] Yoshimi Nakamura,[1] Masanori Koyanagi,[3] Masato Fukui,[3] Hiroyuki Harada,[4] and Yukinari Kato[1,2]

Podoplanin (PDPN) is expressed in type I alveolar cells, kidney podocytes, and lymphatic endothelial cells. We have characterized the PDPNs of various animal species using specific anti-PDPN monoclonal antibodies (mAbs). In this study, we investigated whether these anti-PDPN mAbs cross-react with goat PDPN (gPDPN). Flow cytometry demonstrated that the anti-bovine PDPN mAb PMab-44 (IgG_1, kappa) reacts with gPDPN, which is overexpressed in CHO-K1 cells. Using immunohistochemical analysis, type I alveolar cells of goat lung were strongly detected by PMab-44. These results indicate that PMab-44 is useful for investigating gPDPN.

Keywords: goat podoplanin, PDPN, PMab-44

Introduction

Podoplanin (PDPN) is a type I transmembrane sialoglycoprotein that induces platelet aggregation through the C-type lectin-like receptor-2 (CLEC-2).[1,2] It comprises three platelet aggregation-stimulating (PLAG) domains: PLAG1–3 (EDxxVTPG).[2] Our previous work has demonstrated that PLAG3 is the most important domain for the platelet aggregation activity of human PDPN.[2–4] The most critical domain for PDPN-CLEC-2 interaction remains controversial because these functional assays have been performed using their original anti-PDPN mAbs, the binding affinity of which may vary. Cancer-specific anti-PDPN mAbs should be used for PDPN-targeted therapy[5–9] because PDPN is expressed in many normal tissues.

We have developed many antihuman PDPN monoclonal antibodies (mAbs), including NZ-1.2,[10] LpMab-2,[5,6] LpMab-3,[5] LpMab-7,[5,11] LpMab-9,[5] LpMab-10,[12] LpMab-12,[13] LpMab-13,[14] LpMab-17,[15] LpMab-19,[16] LpMab-21,[17,18] and LpMab-23.[7,8] LpMab-2 and LpMab-23 were categorized as cancer-specific mAbs (CasMabs), and LpMab-3, LpMab-9, LpMab-12, LpMab-19, and LpMab-21 were reported as antiglycopeptide mAbs. PDPN is known to be a specific marker of lymphatic endothelial and type I alveolar cells.[2,19] Therefore, anti-PDPN mAbs are useful for distinguishing lymphatic endothelial cells from vascular endothelial cells or distinguishing type I alveolar cells from type II alveolar cells. In addition, we have characterized PDPNs of various animals using specific anti-PDPN mAbs, such as anti-mouse (PMab-1),[10] anti-rat (PMab-2),[20] anti-rabbit (PMab-32),[21] anti-dog [PMab-38[22] and PMab-48[23]], anti-bovine (PMab-44),[24] and anti-cat (PMab-52)[25] PDPNs. However, to date, anti-goat PDPN (gPDPN) mAbs have not been reported. Therefore, gPDPNs in lymphatic endothelial cells or type I alveolar cells have not been characterized. In this study, we investigated whether our anti-PDPN mAbs from several animal species cross-react with gPDPN using flow cytometry and immunohistochemical analyses.

Materials and Methods

Cell line and goat tissues

Chinese hamster ovary (CHO)-K1 was purchased from the American Type Culture Collection (Manassas, VA). CHO-K1 and transfectants were cultured in RPMI1640 medium (Nacalai Tesque, Inc., Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc., Waltham, MA), 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 25 μg/mL of amphotericin B (Nacalai Tesque, Inc.) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Goat tissues were collected in Nippon Zenyaku Kogyo Co. Ltd. (Fukushima, Japan), fixed in 4%

[1]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan.
[2]New Industry Creation Hatchery Center, Tohoku University, Sendai, Miyagi, Japan.
[3]Zenoaq Resource Co., Ltd., Koriyama, Fukushima, Japan.
[4]Department of Oral and Maxillofacial Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan.

paraformaldehyde phosphate buffer solution (Nacalai Tesque, Inc.), and processed routinely to make paraffin-embedded tissue sections.

Overexpression of gPDPN in CHO-K1 cells

Synthesized DNA (Eurofins Genomics KK, Tokyo, Japan) encoding gPDPN (accession No. XM_005690821.3) plus an N-terminal MAP16 tag (PGTGDGMVPPGIEDKI), which is recognized by an anti-MAP16 tag mAb [PMab-1: the same mAb against MAP tag[26]], was subcloned into a pCAG-Neo vector (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). Plasmids were transfected using Lipofectamine LTX with Plus Reagent (Thermo Fisher Scientific, Inc.). Stable transfectants were selected by limiting dilution and cultivation in a medium containing 0.5 mg/mL of G418 (Nacalai Tesque, Inc.).

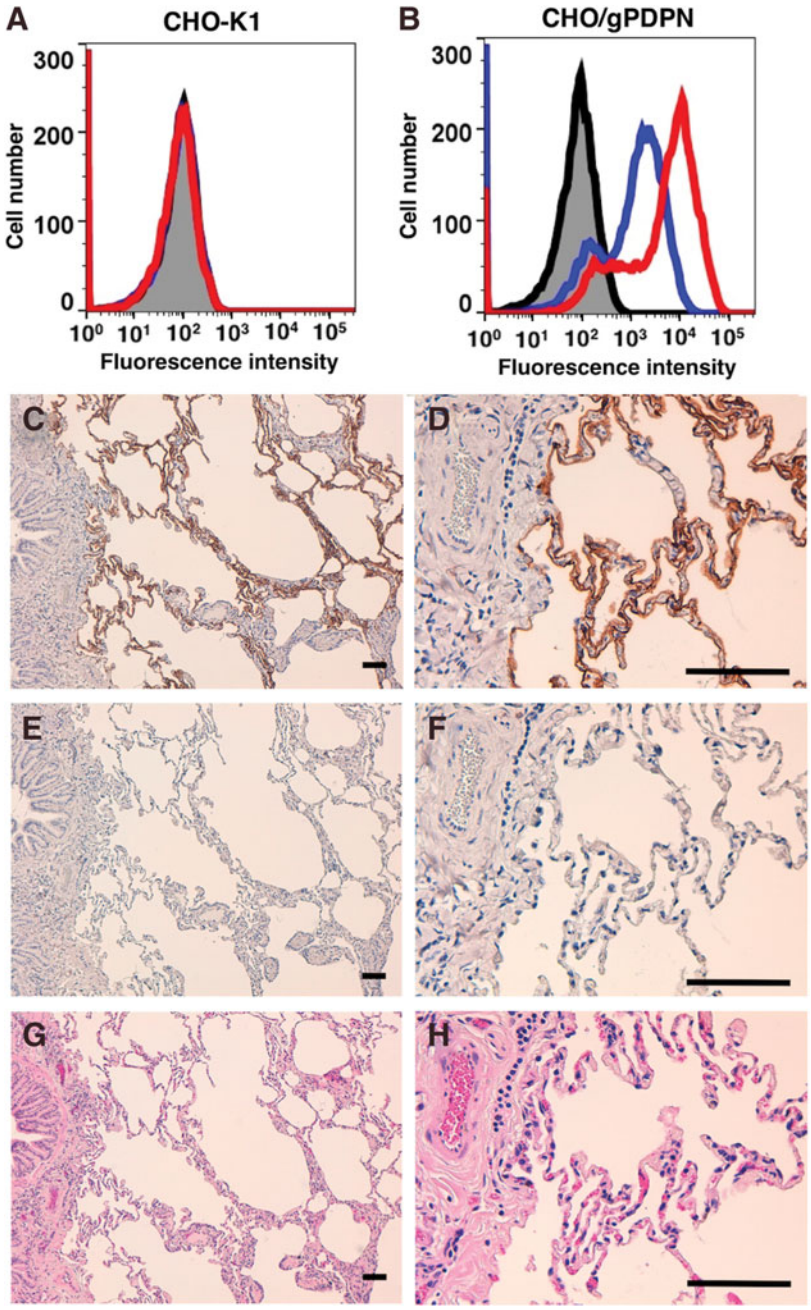

FIG. 1. Detection of gPDPN using PMab-44. **(A, B)** Flow cytometry. PMab-44 at a concentration of 1 μg/mL (blue) or 10 μg/mL (red) was treated with CHO-K1 cells **(A)** or CHO/gPDPN cells **(B)** for 30 min at 4°C, followed by the addition of secondary antibodies. **(C–F)** Immunohistochemical analyses using goat lung tissues. Histological sections of goat lungs were directly autoclaved in citrate buffer for 20 min. After blocking, sections were incubated with 1 μg/mL of PMab-44 **(C, D)** or blocking buffer **(E, F)** and treated with an Envision+ kit. **(G, H)** Hematoxylin and eosin staining. Scale bar = 100 μm. CHO, Chinese hamster ovary; gPDPN, goat podoplanin.

Flow cytometry

CHO/gPDPN and CHO-K1 cells were harvested after a brief exposure to 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.). After washing with 0.1% bovine serum albumin in PBS, the cells were treated with PMab-44 (1 or 10 μg/mL) for 30 min at 4°C, followed by treatment with Alexa Fluor 488-conjugated anti-mouse IgG (1:1000; Cell Signaling Technology, Inc., Danvers, MA). Fluorescence data were acquired using a Cell Analyzer SA3800 (Sony Corporation, Tokyo, Japan).

Immunohistochemical analyses

Histological sections (4-μm thick) of goat tissues were directly autoclaved in citrate buffer (pH 6.0; Nichirei Biosciences, Inc., Tokyo, Japan) for 20 min. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), sections were incubated with PMab-44 (1 μg/mL) for 1 h at room temperature and treated using an Envision+ kit (Agilent Technologies, Inc., Santa Clara, CA) for 30 min. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (Agilent Technologies, Inc.) for 2 min, and sections were counterstained with hematoxylin (Fujifilm Wako Pure Chemical Corporation).

Results and Discussion

We previously developed a mouse anti-bovine PDPN mAb, PMab-44 (IgG_1, kappa), which specifically detects bovine PDPN in flow cytometry and immunohistochemistry.[24] Furthermore, a series of deletion or point mutants of bovine PDPN were used for investigating the binding epitopes of PMab-44 using flow cytometry and Western blotting.[27] Platelet aggregation-stimulating domain 3 (PLAG3) of bovine PDPN was identified as a critical epitope of PMab-44. The comparison of amino acid sequences revealed that 87% homology is observed between bovine PDPN and gPDPN (Supplementary Fig. S1). Therefore, we investigated whether PMab-44 cross-reacts with gPDPN in this study.

We first produced a gPDPN-stable transfectant using CHO-K1 cells using N-terminal MAP16 tag. In flow cytometry, PMab-44 did not react with CHO-K1 cells (Fig. 1A), but reacted with CHO/gPDPN in a dose-dependent manner (Fig. 1B). The other anti-PDPN mAbs, such as anti-rat (PMab-2),[20] anti-rabbit (PMab-32),[21] anti-dog [PMab-38[22] and PMab-48[23]], and anti-cat PDPN [PMab-52[25]], did not react with CHO/gPDPN (data not shown). These results indicate that PMab-44 is useful for detecting gPDPN.

Next, we investigated gPDPN expression using immunohistochemistry. Previous immunohistochemical studies have reported that PMab-44 reacts with bovine tissues.[24] Similarly, PMab-44 strongly stained type I alveolar cells of goat lung in a membrane-staining pattern (Fig. 1C, D). Conversely, lymphatic endothelial cells of goat colon were not stained by PMab-44 in this study (data not shown), indicating that PMab-44 reactivity in goat exhibits tissue specificity.

Taken together, PMab-44 is useful for detecting gPDPN in flow cytometry and immunohistochemical analyses. Further studies are necessary to show that PMab-44 can detect gPDPN in other normal goat tissues.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Yukinari Kato
New Industry Creation Hatchery Center
Tohoku University
2-1, Seiryo-machi, Aoba-ku
Sendai
Miyagi 980-8575
Japan

E-mail: yukinarikato@med.tohoku.ac.jp

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