MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 38, Number 6, 2019

DOI: 10.1089/mab.2019.0038

Short Communication

PMab-241 Specifically Detects Bear Podoplanin of Lymphatic Endothelial Cells in the Lung of Brown Bear

Junko Takei,[1] Shinji Yamada,[1] Satoru Konnai,[2,3] Tsuyoshi Ishinazaka,[4] Michito Shimozuru,[5] Mika K. Kaneko,[1] and Yukinari Kato[1,6]

Podoplanin (PDPN)/T1alpha is utilized as a specific marker of lymphatic endothelial cells or type I alveolar cells of lung. Therefore, sensitive and specific monoclonal antibodies (mAbs) detecting PDPN are necessary for immunohistochemical analyses, especially using formalin-fixed paraffin-embedded tissues. Recently, we developed an anti-bear PDPN (bPDPN) mAb, PMab-247, which is useful for immunohistochemical analyses to detect both lymphatic endothelial cells and type I alveolar cells of lung. However, it is difficult to distinguish lymphatic endothelial cells from type I alveolar cells in the bear lung. In this study, we showed that a novel anti-bPDPN mAb, PMab-241 stained only lymphatic endothelial cells, not type I alveolar cells of the lung in immunohistochemical analyses. These findings suggest that PMab-241 could be useful for staining lymphatic endothelial cells specifically in the bear lung tissues.

Keywords: bear podoplanin, PDPN, type I alveolar cells, lymphatic endothelial cells, PMab-241

Introduction

Podoplanin (PDPN) is a type I transmembrane sialoglycoprotein,[1] and is also known as T1alpha [2]/gp44 [3]/Aggrus,[4] which induces platelet aggregation (PLAG) through C-type lectin-like receptor-2 (CLEC-2).[5,6] The sialic acid of the PLAG-stimulating domain or the PLAG-like domain of PDPN is known to be important for PDPN-induced PLAG.[7,8]

PDPN is expressed in several normal cells, including pulmonary type I alveolar cells and lymphatic endothelial cells of every organ.[1] PDPN is used to distinguish lymphatic endothelial cells from vascular endothelial cells in pathophysiological studies.[9] However, PDPN is expressed in both type I alveolar cells and lymphatic endothelial cells in the lung; therefore, it might be difficult to distinguish lymphatic endothelial cells from type I alveolar cells in the lung tissues.

Materials and Methods

Cell lines

CHO-K1 was obtained from the American Type Culture Collection (ATCC; Manassas, VA). CHO/bear PDPN (bPDPN) was previously established.[10] CHO-K1 and CHO/bPDPN were cultured in a Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), supplemented with 10% of 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.). The cells were grown in an incubator at 37°C with humidity and 5% CO_2 and 95% air atmosphere.

Flow cytometry

The cells were harvested after a brief exposure to 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.). The cells were washed with 0.1% bovine serum albumin in phosphate-buffered saline (PBS) and treated with primary monoclonal antibodies (mAbs) for 30 minutes at 4°C. Thereafter, the cells were treated with Alexa Fluor 488-conjugated anti-mouse IgG (1:2000; Cell Signaling Technology, Inc., Danvers, MA). Then, fluorescence data were collected using the SA3800 Cell Analyzer (Sony Corp., Tokyo, Japan).

[1]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan.
[2]Laboratory of Infectious Diseases, Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.
[3]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.
[4]Shiretoko Nature Foundation, Shari, Hokkaido, Japan.
[5]Laboratory of Wildlife Biology and Medicine, Department of Environmental Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.
[6]New Industry Creation Hatchery Center, Tohoku University, Sendai, Miyagi, Japan.

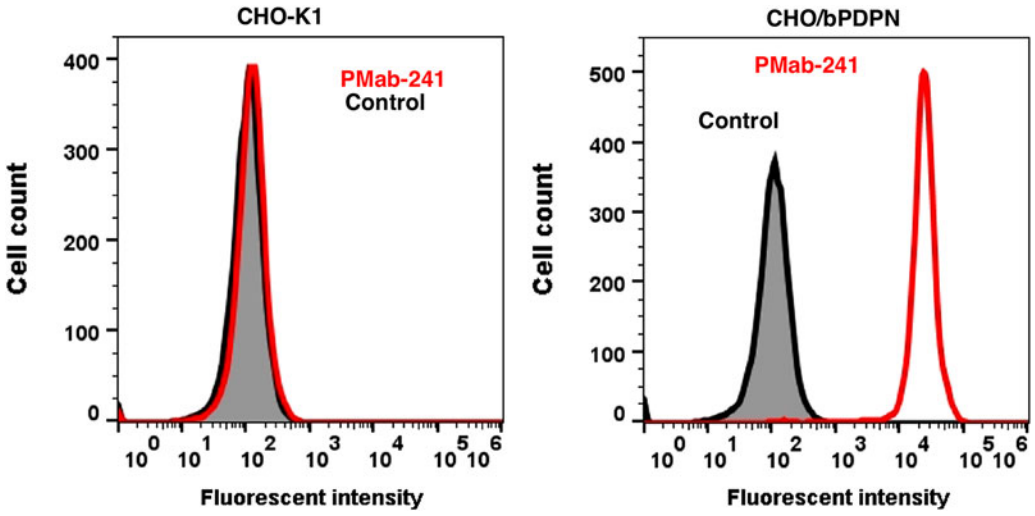

FIG. 1. Detection of bPDPN by flow cytometry using PMab-241. CHO/bPDPN and CHO-K1 were treated with PMab-241 (red line) at a concentration of 1 μg/mL or 0.1% bovine serum albumin in phosphate-buffered saline (gray) for 30 minutes, followed by incubation with secondary antibodies. bPDPN, bear podoplanin.

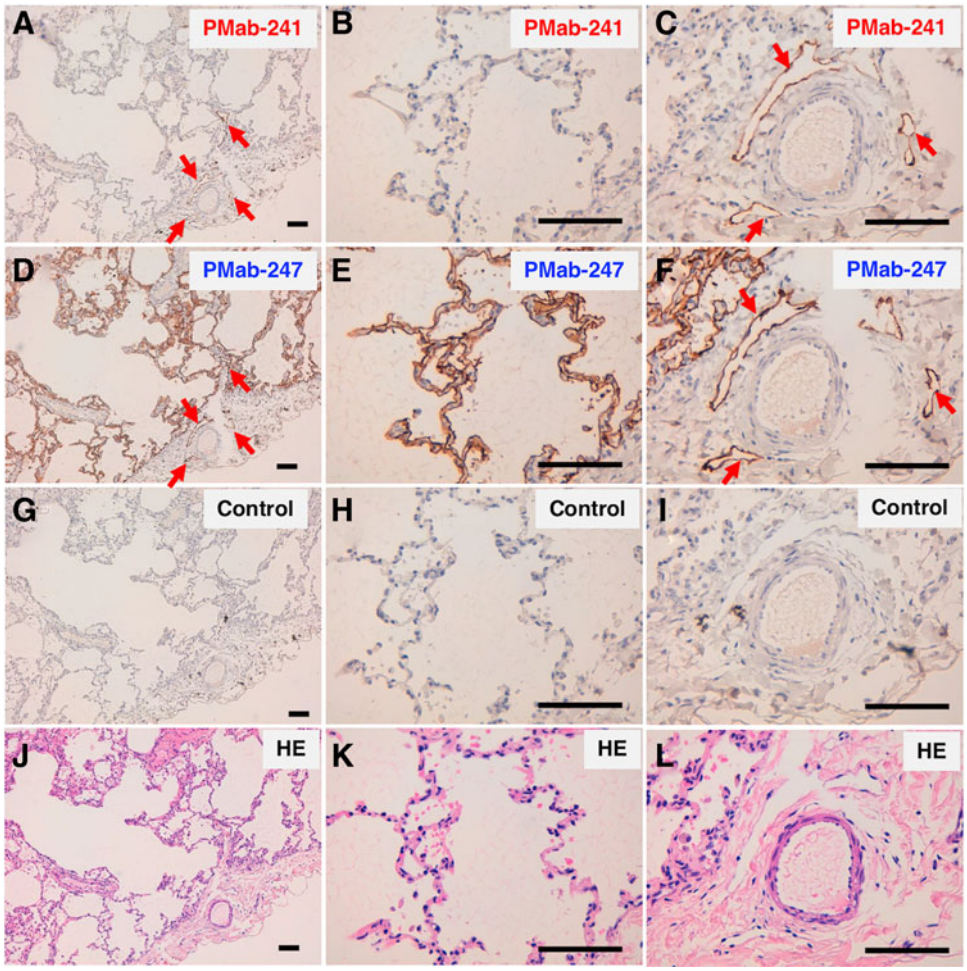

FIG. 2. Immunohistochemical analyses for the bear lung. The bear lung sections were incubated with 1 μg/mL of PMab-241 **(A–C)**, PMab-247 **(D–F)** or with blocking buffer **(G–I)**, followed by that with the Envision+ Kit. **(J– L)** Hematoxylin and eosin staining. Scale bar = 100 μm. Red arrows indicate lymphatic endothelial cells.

Immunohistochemical analyses

Four brown bear (*Ursus arctos*) tissues were collected after autopsy at Shiretoko Nature Foundation, fixed in 10% neutral-buffered formalin, and routinely processed to make paraffin-embedded tissue sections. Histological sections (4-μm thick) were directly autoclaved in citrate buffer (pH 6.0; Nichirei Biosciences, Inc., Tokyo, Japan) for 20 minutes. Then, sections were blocked using the SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), incubated with PMab-241 or PMab-247 (1 μg/mL) for 1 hour at room temperature, and treated with the Envision+ Kit for mouse (Agilent Technologies, Inc., Santa Clara, CA) for 30 minutes. Color was developed using 3,3′-diaminobenzidine tetrahydrochloride (Agilent Technologies, Inc.) for 2 minutes, and counterstaining was performed using hematoxylin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Results and Discussion

Although we have already established an anti-bPDPN mAb (PMab-247), which is sensitive and specific for immunohistochemical analyses,[10] it is difficult to distinguish lymphatic endothelial cells from type I alveolar cells in the bear lung using PMab-247.[11] In this study, we selected a novel anti-bPDPN mAb (PMab-241) of IgG_{2b} subclass from previously established anti-bPDPN mAbs.[10] PMab-241 reacted with CHO/bPDPN, not parental CHO-K1 cells in flow cytometry (Fig. 1).

We performed immunohistochemical analyses against the bear lungs using two anti-bPDPN mAbs, PMab-241 and PMab-247. PMab-241 stained lymphatic endothelial cells of the bear lung (Fig. 2A, C), but not type I alveolar cells (Fig. 2A, B) although PMab-247 stained both lymphatic endothelial cells (Fig. 2D, F) and type I alveolar cells (Fig. 2D, E). We stained the lungs from the other three bears using PMab-241 and PMab-247, and obtained the similar staining results (Supplementary Figs. S1–S3). These results indicate that PMab-241 could be a specific marker of lymphatic endothelial cells in the bear lung.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This research was supported in part by AMED under grant nos. JP19am0401013, JP19am0101078, and JP19ae0101028 (Y.K.), and by JSPS KAKENHI grant nos. 17K07299 (M.K.K.) and 19K07705 (Y.K.).

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3

References

1. Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, Schaffner G, and Kerjaschki D: Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. Am J Pathol 1997;151:1141–1152.
2. Meneghetti A, Cardoso WV, Brody JS, and Williams MC: Epithelial marker genes are expressed in cultured embryonic rat lung and *in vivo* with similar spatial and temporal patterns. J Histochem Cytochem 1996;44:1173–1182.
3. Toyoshima M, Nakajima M, Yamori T, and Tsuruo T: Purification and characterization of the platelet-aggregating sialoglycoprotein gp44 expressed by highly metastatic variant cells of mouse colon adenocarcinoma 26. Cancer Res 1995;55:767–773.
4. Kato Y, Fujita N, Kunita A, Sato S, Kaneko M, Osawa M, and Tsuruo T: Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem 2003;278:51599–51605.
5. Suzuki-Inoue K, Kato Y, Inoue O, Kaneko MK, Mishima K, Yatomi Y, Yamazaki Y, Narimatsu H, and Ozaki Y: Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. J Biol Chem 2007;282:25993–26001.
6. Kato Y, Kaneko MK, Kunita A, Ito H, Kameyama A, Ogasawara S, Matsuura N, Hasegawa Y, Suzuki-Inoue K, Inoue O, Ozaki Y, and Narimatsu H: Molecular analysis of the pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. Cancer Sci 2008;99:54–61.
7. Kaneko M, Kato Y, Kunita A, Fujita N, Tsuruo T, and Osawa M: Functional sialylated O-glycan to platelet aggregation on Aggrus (T1alpha/podoplanin) molecules expressed in Chinese Hamster Ovary cells. J Biol Chem 2004; 279:38838–38843.
8. Kaneko MK, Kato Y, Kameyama A, Ito H, Kuno A, Hirabayashi J, Kubota T, Amano K, Chiba Y, Hasegawa Y, Sasagawa I, Mishima K, and Narimatsu H: Functional glycosylation of human podoplanin: Glycan structure of platelet aggregation-inducing factor. FEBS Lett 2007;581: 331–336.
9. Jung E, Gardner D, Choi D, Park E, Jin Seong Y, Yang S, Castorena-Gonzalez J, Louveau A, Zhou Z, Lee GK, Perrault DP, Lee S, Johnson M, Daghlian G, Lee M, Jin Hong Y, Kato Y, Kipnis J, Davis MJ, Wong AK, and Hong YK: Development and characterization of a novel Prox1-EGFP lymphatic and Schlemm's canal reporter rat. Sci Rep 2017; 7:5577.
10. Furusawa Y, Takei J, Sayama Y, Yamada S, Kaneko MK, and Kato Y: Development of an anti-bear podoplanin monoclonal antibody PMab-247 for immunohistochemical analysis. Biochem Biophys Rep 2019;18:100644.
11. Takei J, Furusawa Y, Yamada S, Nakamura T, Sayama Y, Sano M, Konnai S, Kobayashi A, Harada H, Kaneko MK, and Kato Y: PMab-247 detects bear podoplanin in immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2019;38:171–174.

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

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

Received: August 21, 2019
Accepted: October 15, 2019