MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 38, Number 1, 2019

DOI: 10.1089/mab.2018.0038

PMab-210: A Monoclonal Antibody Against Pig Podoplanin

Yoshikazu Furusawa,[1–3] Shinji Yamada,[1] Shunsuke Itai,[1,4] Masato Sano,[1] Takuro Nakamura,[1] Miyuki Yanaka,[1] Masato Fukui,[3] Hiroyuki Harada,[4] Takuya Mizuno,[5] Yusuke Sakai,[6] Masaki Takasu,[7] Mika K. Kaneko,[1] and Yukinari Kato[1,2]

Podoplanin (PDPN) is a type I transmembrane glycoprotein that is expressed in normal tissues, including renal corpuscles and type I lung alveolar cells. Monoclonal antibodies (mAbs) against human, mouse, rat, rabbit, dog, cat, and bovine PDPNs have already been established; however, antipig PDPN (pPDPN) mAbs have not. We therefore immunized mice with pPDPN-overexpressing Chinese hamster ovary (CHO)-K1 cells (CHO/pPDPN), and screened hybridomas, which are producing anti-pPDPN mAbs. One of mAbs, PMab-210 (an IgG_1, kappa), was able to specifically detect CHO/pPDPN cells by flow cytometry and detect pPDPN by Western blot analysis. Furthermore, PMab-210 strongly stained type I lung alveolar cells and weakly stained renal corpuscles by immunohistochemistry. PMab-210 is expected to be useful in investigating the function of pPDPN.

Keywords: pig podoplanin, PDPN, PMab-210

Introduction

Podoplanin (PDPN)/T1α/Aggrus is a type I transmembrane glycoprotein expressed in normal tissues, including renal corpuscles, type I lung alveolar cells, and lymphatic endothelial cells.[1,2] PDPN induces platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2),[1,3–9] and the interaction between PDPN on lymphatic endothelial cells and CLEC-2 on platelets facilitates embryonic blood/lymphatic vessel separation.[10] PDPN is also expressed in human fetal ribs and in chondrocytes of the proliferative and hypertrophic regions of the growth plate.[11] The expression of human PDPN has been reported in several malignant tumors, such as esophageal cancers,[12] malignant mesotheliomas,[13,14] lung cancers,[15] testicular tumors,[16] osteosarcomas,[17–19] chondrosarcomas,[18] oral squamous cell carcinomas,[20] and malignant brain tumors.[21–24] PDPN expression is also associated with malignant progression and cancer metastasis.[6,21,25]

We have previously developed monoclonal antibodies (mAbs) against human,[26] mouse,[26] rat,[27] rabbit,[28] dog,[29] cat,[30] and bovine[31] PDPNs. However, antipig PDPN (pPDPN) mAbs have not yet been produced. Sensitive and specific mAbs against pPDPN are needed to investigate its expression and function. In this study, we therefore immunized mice with CHO/pPDPN cells and established cell lines producing anti-pPDPN mAbs.

Materials and Methods

Cell lines

Chinese hamster ovary (CHO)-K1 and P3U1 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The pig kidney cell line, PK-15, was obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). pPDPN bearing an N-terminal PA16 tag (PA16-pPDPN) was inserted into a pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The PA16 tag consists of 16 amino acids (GLEGGVAMPG AEDDVV).[32] CHO-K1 cells were transfected with pCAG-Ble/PA16-pPDPN using Lipofectamine LTX with Plus Reagent (Thermo Fisher Scientific, Inc., Waltham, MA). Stable transfectants were selected by limiting dilution and cultivated in a medium containing 0.5 mg/mL zeocin (InvivoGen, San Diego, CA). CHO-K1, CHO/pPDPN, and P3U1

[1]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan.
[2]New Industry Creation Hatchery Center, Tohoku University, Sendai, Japan.
[3]ZENOAQ RESOURCE CO., LTD., Koriyama, Japan.
[4]Department of Oral and Maxillofacial Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Japan.
[5]Laboratory of Molecular Diagnostics and Therapeutics, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan.
[6]Laboratory of Veterinary Pathology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan.
[7]Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan.

cells were cultured in RPMI 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), and PK-15 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Nacalai Tesque, Inc.). All media were supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.), 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.

Hybridoma production

Female BALB/c mice (6-week old) were purchased from CLEA Japan (Tokyo, Japan). Animals were housed under specific pathogen-free conditions. The Animal Care and Use Committee of Tohoku University approved all the animal experiments described here. BALB/c mice were immunized against CHO/pPDPN cells (1×10^8), which were administered intraperitoneally (i.p.) together with Imject Alum (Thermo Fisher Scientific, Inc.). After an additional three immunizations, a final booster injection was administered i.p. 2 days before spleen cells were harvested. The spleen cells were then fused with P3U1 cells using PEG1500 (Roche Diagnostics, Indianapolis, IN), and the hybridomas were grown in RPMI medium supplemented with hypoxanthine, aminopterin, and thymidine for selection (Thermo Fisher Scientific, Inc.). The culture supernatants were screened by flow cytometry.

Flow cytometry

Cells were harvested after brief exposure to 0.25% trypsin/1 mM EDTA (Nacalai Tesque, Inc.). After washing with 0.1% bovine serum albumin/phosphate-buffered saline (PBS), the cells were treated with primary mAbs for 30 minutes at 4°C, followed by treatment with Alexa Fluor 488-conjugated antimouse IgG (1:2000; Cell Signaling Technology, Inc., Danvers, MA) or Oregon green antirat IgG (1:2000; Thermo Fisher Scientific Inc.). Fluorescence data were collected using SA3800 Cell Analyzers (Sony Corp., Tokyo, Japan).

Western blot analysis

Cell lysates (10 μg) were boiled in sodium dodecyl sulfate sample buffer (Nacalai Tesque, Inc.). The proteins were electrophoresed on 5%–20% polyacrylamide gels (FUJIFILM Wako Pure Chemical Corporation), and then transferred onto a polyvinylidene difluoride membrane (Merck KGaA, Darmstadt, Germany). After blocking with 4% skim milk (Nacalai Tesque, Inc.), the membrane was incubated with primary mAbs, and subsequently with peroxidase-conjugated antimouse IgG (1:1000; Agilent Technologies, Santa Clara, CA) or antirat IgG (1:10,000; Sigma-Aldrich Corp., St. Louis, MO). Bands were visualized with ImmunoStar LD (FUJIFILM Wako Pure Chemical Corporation) using a Sayaca-Imager (DRC Co. Ltd., Tokyo, Japan).

Determination of binding affinity using flow cytometry

CHO/pPDPN or PK-15 (2×10^5 cells) was suspended in 100 μL of serially diluted PMab-210, followed by addition of Alexa Fluor 488-conjugated antimouse IgG (1:200; Cell Signaling Technology, Inc.). Fluorescence data were collected using EC800 Cell Analyzer (Sony Corp.). The dissociation constants (K_D) were obtained by fitting the binding isotherms to built-in one-site binding models in GraphPad PRISM 6 (GraphPad Software, Inc., La Jolla, CA).

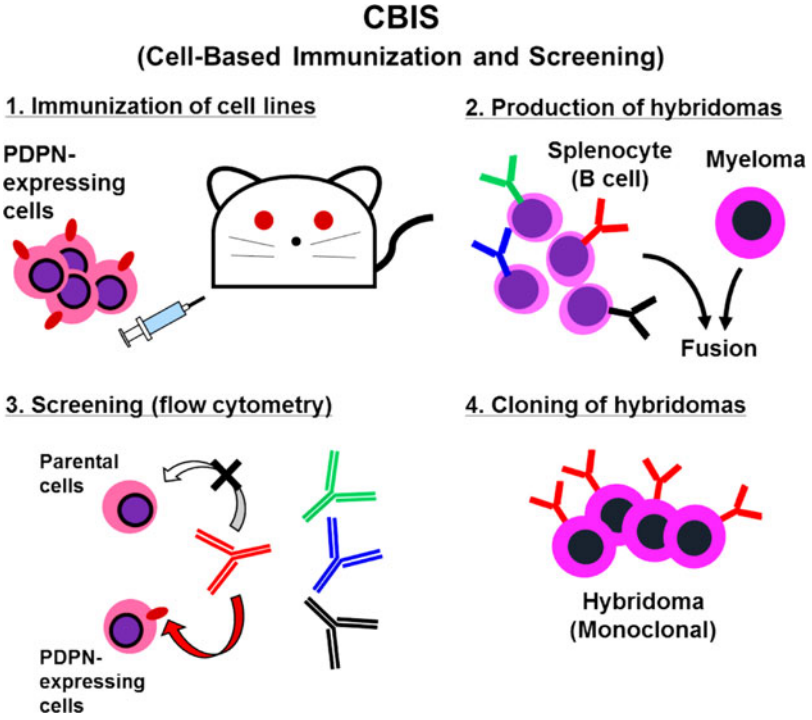


FIG. 1. Schematic illustration of CBIS. Stable transfectants expressing the protein of interest are used as an immunogen with no purification procedure. The selection of hybridomas secreting specific mAbs is performed by flow cytometry using the parental and transfectant cells. CBIS, Cell-Based Immunization and Screening; mAbs, monoclonal antibodies.

Immunohistochemical analyses

Pig tissues were collected at autopsy at Yamaguchi University and fixed in 10% neutral-buffered formalin. Micromini pig (Fuji Micra, Inc., Shizuoka, Japan) tissues were collected at autopsy at Gifu University and fixed in 4% paraformaldehyde. Tissues were processed routinely to produce 4 μm paraffin-embedded tissue sections, which were directly autoclaved in citrate buffer (pH 6.0; Nichirei Biosciences, Inc., Tokyo, Japan) or EnVision FLEX Target Retrieval Solution, High pH (Agilent Technologies, Inc.) for 20 minutes. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), sections were incubated with PMab-210 (10 or 20 μg/mL) for 1 hour at room temperature and treated using an Envision+ Kit (Agilent Technologies, Inc.) for 30 minutes. Color was developed using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Agilent Technologies, Inc.) for 2 minutes, and counterstaining was performed with hematoxylin (FUJIFILM Wako Pure Chemical Corporation).

Results and Discussion

To produce sensitive and specific anti-pPDPN mAbs for immunohistochemical analysis of paraffin-embedded tissue sections, we first immunized mice with two different synthetic pPDPN-derived peptides; however, we were unsuccessful (data not shown). We therefore instead employed a Cell-Based Immunization and Screening (CBIS) method for producing anti-pPDPN mAbs. We have previously successfully utilized a CBIS method to establish mAbs against various types of membrane proteins such as CD44,[32] CD133,[33] PD-L1,[34] and cat PDPN (cPDPN),[30] so here we immunized one mouse with pPDPN-overexpressing CHO-K1 (CHO/pPDPN) cells using the immunization and screening procedure depicted in Figure 1. The hybridomas were seeded into and cultured in 96-well plates, and supernatants were used to label and screen cells by flow cytometry. CHO/pPDPN cells were screened to identify potential anti-pPDPN antibodies, whereas CHO-K1 cells were screened to exclude antibodies against CHO-K1 cells antigens. PK-15 cells were also used to identify antibodies that reacted with the endogenous pig protein. Screening identified strong signals against CHO/pPDPN cells and weak or no signals against CHO-K1 cells in 44 of 480 wells (9.2%). Among these 44 wells, seven produced strong signals against PK-15 cells and were negative against CHO-K1 cells. PMab-210 (IgG_1, kappa) was finally established by limiting dilution from one of these wells.

PMab-210 recognized CHO/pPDPN but did not react with CHO-K1, as assessed by flow cytometry (Fig. 2). It also reacted with the PK-15 pig kidney cell line, indicating that it could recognize endogenous pPDPN. PMab-210 did not react with human, mouse, rat, rabbit, dog, cat, or bovine PDPNs (Fig. 3). Furthermore, it did not react with horse, Tasmanian devil, tiger, alpaca, bear, goat, sheep, or whale PDPNs (Supplementary Fig. S1), indicating that PMab-210 is specific to pPDPN. Flow cytometry was next used to perform a kinetic analysis of the interaction of PMab-210 with CHO/pPDPN and PK-15 cells. The dissociation constant (K_D) of PMab-210 for CHO/pPDPN and PK-15 cells was determined to be 4.0×10^{-8} M and 4.4×10^{-8} M, respectively, indicating a moderate affinity for either cell type.

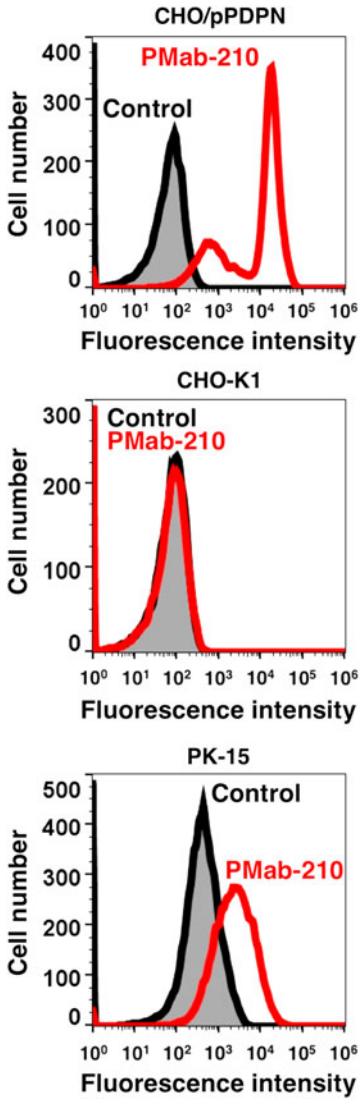
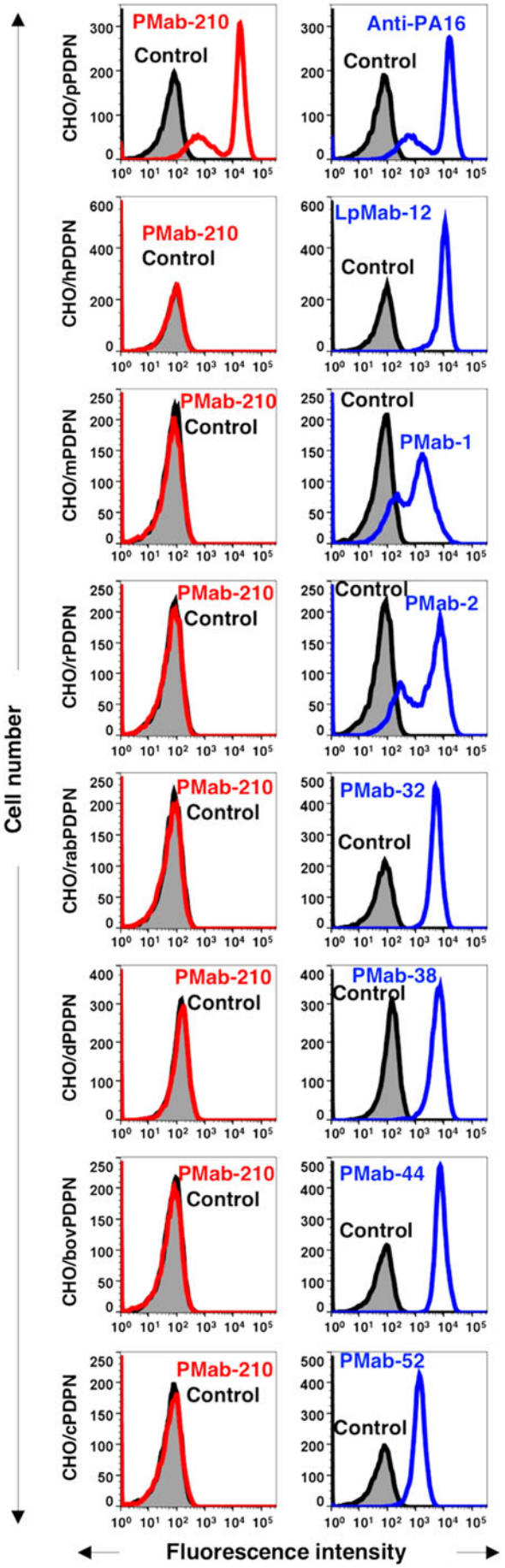


FIG. 2. Detection of pPDPN by flow cytometry using PMab-210. CHO/pPDPN, CHO-K1, and PK-15 cells were treated with PMab-210 (red line) at a concentration of 10 μg/mL or control (gray) for 30 minutes, followed by the incubation with secondary antibodies. CHO, Chinese hamster ovary; pPDPN, pig podoplanin.

PMab-210 was able to identify pPDPN as a 40 kDa band in the Western blot of CHO/pPDPN cells (Fig. 4), but did not detect it in PK-15 cells, possibly due to low expression of pPDPN in PK-15 cells or a low affinity of PMab-210 against PK-15 cells. An anti-PA16 tag mAb (NZ-1) detected two strong bands in CHO/pPDPN cells: the upper is reported to be highly glycosylated form and the lower one is unglycosylated.[3,24] In contrast, PMab-210 recognized only the upper glycosylated pPDPN, suggesting that the mAb may recognize glycosylated pPDPN more selectively. Our previous studies have similarly found that antihuman PDPN (hPDPN) mAbs such as LpMab-3,[35] LpMab-9,[36] LpMab-19,[37] and LpMab-21[38] also detect only glycosylated hPDPN. It would be interesting to identify the specific epitope of PMab-210 in the future.

When used in immunohistochemistry, PMab-210 strongly stained type I pulmonary alveolar cells of pigs (Fig. 5) and



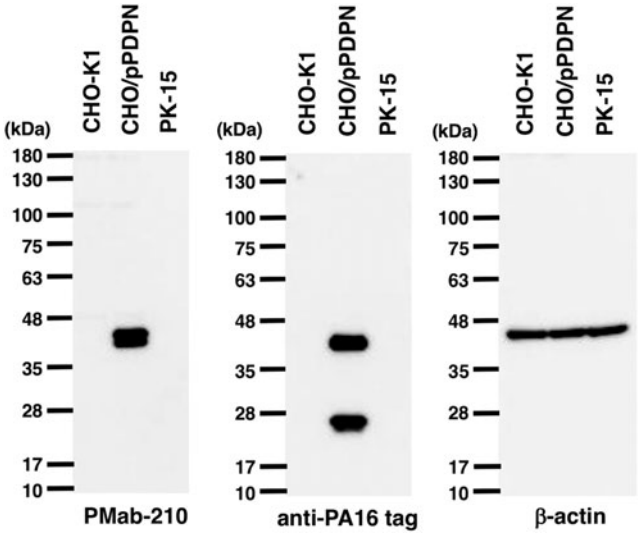


FIG. 4. Western blot analysis. Cell lysates of CHO-K1, CHO/pPDPN, and PK-15 (10 μg) were electrophoresed and transferred onto a PVDF membrane. The membrane was incubated with 10 μg/mL of PMab-210, 1 μg/mL of anti-PA tag (NZ-1), or 1 μg/mL of anti-β-actin, and subsequently with peroxidase-conjugated antimouse or -rat IgG. PVDF, polyvinylidene difluoride.

micromini pigs (Fig. 6). Furthermore, PMab-210 weakly stained renal corpuscles of pigs (Fig. 7) and micromini pigs (Fig. 8), indicating that the mAb is useful for the detection of PDPN by immunohistochemistry. PDPN is known to be a specific lymphatic endothelial cell marker; however, PMab-210 could not detect any lymphatic endothelial cells in lungs, kidneys, or colons of pigs or micromini pigs (data not shown). We could not obtain primary cells or established cell lines of pig lymphatic endothelial cells in this study, and so we could not determine whether PMab-210 could not react with lymphatic endothelial cells in paraffin sections or whether pig lymphatic endothelial cells exhibit tissue-specific glycosylation and so do not carry the PMab-210 epitope.

Taken together, these data demonstrate that we have established an anti-pPDPN mAb, PMab-210, which is applicable for use in flow cytometry, Western blotting, and immunohistochemical analyses. The epitope of PMab-210 should be determined in a future study to further clarify the sensitivity and specificity of PMab-210 against pPDPN. This mAb should prove useful for elucidating the pathophysiological functions of pPDPN in the future studies.

FIG. 3. Specific detection of pPDPN by flow cytometry using PMab-210. CHO/pPDPN, CHO/hPDPN, CHO/mPDPN, CHO/rPDPN, CHO/rabPDPN, CHO/dPDPN, CHO/bovPDPN, and CHO/cPDPN cells were treated with PMab-210 (red line) at a concentration of 10 μg/mL or control (gray) for 30 minutes, followed by the addition of secondary antibodies. The same cells were also treated with positive control (blue line) at a concentration of 1 μg/mL or control (gray) for 30 minutes, followed by the addition of secondary antibodies.

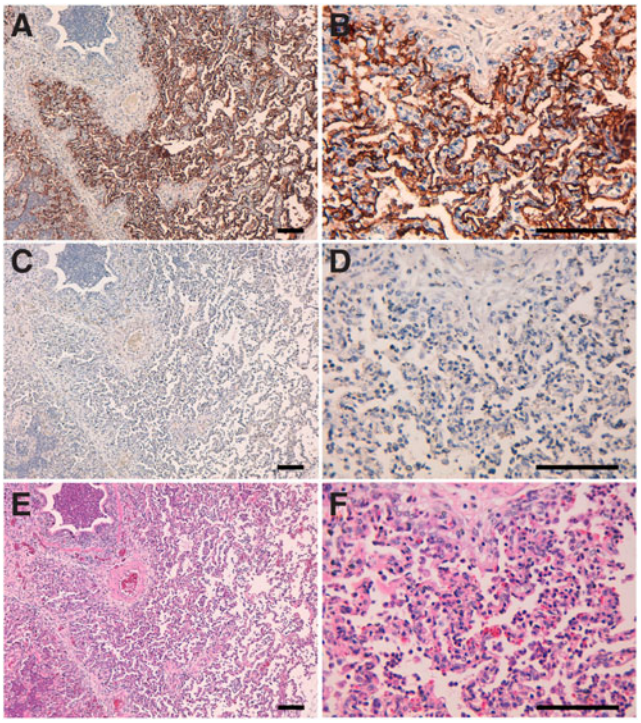


FIG. 5. Immunohistochemical analyses using pig lung tissue. Histological sections were incubated with 10 μg/mL of PMab-210 **(A, B)** or with blocking buffer **(C, D)**, followed by an Envision+ Kit. **(E, F)** Hematoxylin and eosin staining. Scale bar = 100 μm.

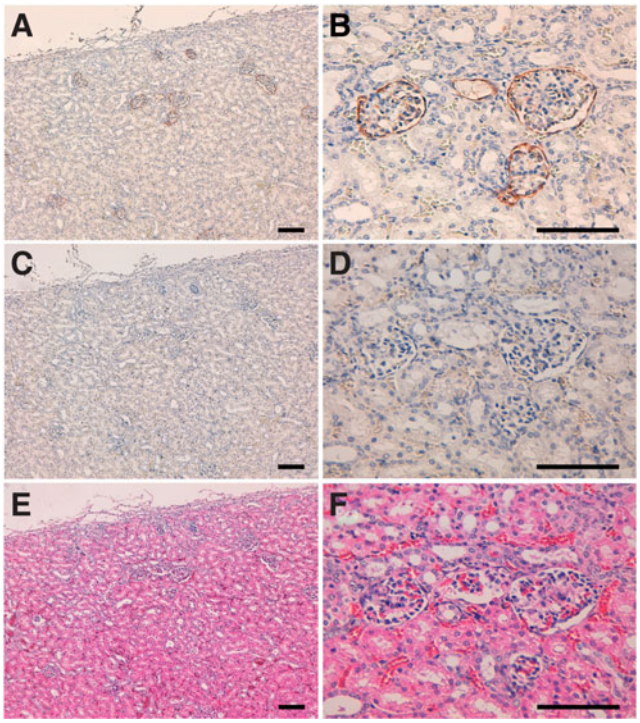


FIG. 7. Immunohistochemical analyses using pig kidneys. Histological sections of pig kidney tissue were incubated with 10 μg/mL of PMab-210 **(A, B)** or with blocking buffer **(C, D)**, followed by an Envision+ Kit. **(E, F)** Hematoxylin and eosin staining. Scale bar = 100 μm.

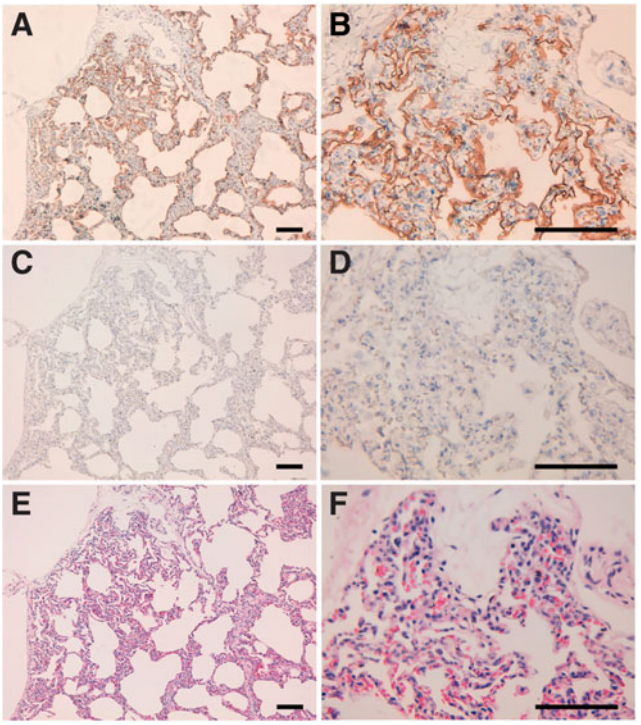


FIG. 6. Immunohistochemical analyses using micromini pig lungs. Histological sections of micromini pig lung tissue were incubated with 10 μg/mL of PMab-210 **(A, B)** or with blocking buffer **(C, D)**, followed by an Envision+ Kit. **(E, F)** Hematoxylin and eosin staining. Scale bar = 100 μm.

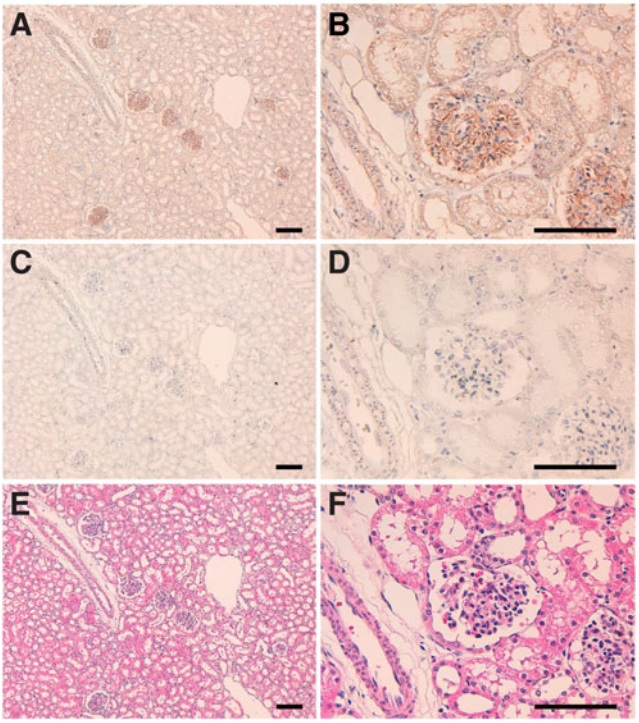


FIG. 8. Immunohistochemical analyses using micromini pig kidneys. Histological sections of the micromini pig kidney tissue were incubated with 20 μg/mL of PMab-210 **(A, B)** or with blocking buffer **(C, D)**, followed by an Envision+ Kit. **(E, F)** Hematoxylin and eosin staining. Scale bar = 100 μm.

Acknowledgments

We thank Saori Handa, Kayo Hisamatsu, and Yoshimi Nakamura for their excellent technical assistance. This research was supported in part by AMED under grant numbers JP18am0101078 (Y.K.), JP18am0301010 (Y.K.), and JP18ae0101028 (Y.K.), and by JSPS KAKENHI grant number 17K07299 (M.K.K.) and grant number 16K10748 (Y.K.).

Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1

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. Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K, and Kerjaschki D: Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: Podoplanin as a specific marker for lymphatic endothelium. Am J Pathol 1999;154:385–394.
3. 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.
4. Kaneko MK, Kato Y, Kitano T, and Osawa M: Conservation of a platelet activating domain of Aggrus/podoplanin as a platelet aggregation-inducing factor. Gene 2006;378:52–57.
5. Martin-Villar E, Scholl FG, Gamallo C, Yurrita MM, Munoz-Guerra M, Cruces J, and Quintanilla M: Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. Int J Cancer 2005;113:899–910.
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 MK, Kunita A, Abe S, Tsujimoto Y, Fukayama M, Goto K, Sawa Y, Nishioka Y, and Kato Y: Chimeric anti-podoplanin antibody suppresses tumor metastasis through neutralization and antibody-dependent cellular cytotoxicity. Cancer Sci 2012;103:1913–1919.
8. 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.
9. Nagae M, Morita-Matsumoto K, Kato M, Kaneko MK, Kato Y, and Yamaguchi Y: A platform of C-type lectin-like receptor CLEC-2 for binding O-glycosylated podoplanin and nonglycosylated rhodocytin. Structure 2014;22:1711–1721.
10. Bertozzi CC, Schmaier AA, Mericko P, Hess PR, Zou Z, Chen M, Chen CY, Xu B, Lu MM, Zhou D, Sebzda E, Santore MT, Merianos DJ, Stadtfeld M, Flake AW, Graf T, Skoda R, Maltzman JS, Koretzky GA, and Kahn ML: Platelets regulate lymphatic vascular development through CLEC-2-SLP-76 signaling. Blood 2010;116:661–670.
11. Smith SM, and Melrose J: Podoplanin is expressed by a sub-population of human foetal rib and knee joint rudiment chondrocytes. Tissue Cell 2011;43:39–44.
12. Schoppmann SF, Jesch B, Riegler MF, Maroske F, Schwameis K, Jomrich G, and Birner P: Podoplanin expressing cancer associated fibroblasts are associated with unfavourable prognosis in adenocarcinoma of the esophagus. Clin Exp Metastasis 2013;30:441–446.
13. Kimura N, and Kimura I: Podoplanin as a marker for mesothelioma. Pathol Int 2005;55:83–86.
14. Abe S, Morita Y, Kaneko MK, Hanibuchi M, Tsujimoto Y, Goto H, Kakiuchi S, Aono Y, Huang J, Sato S, Kishuku M, Taniguchi Y, Azuma M, Kawazoe K, Sekido Y, Yano S, Akiyama S, Sone S, Minakuchi K, Kato Y, and Nishioka Y: A novel targeting therapy of malignant mesothelioma using anti-podoplanin antibody. J Immunol 2013;190:6239–6249.
15. Kato Y, Kaneko M, Sata M, Fujita N, Tsuruo T, and Osawa M: Enhanced expression of Aggrus (T1alpha/podoplanin), a platelet-aggregation-inducing factor in lung squamous cell carcinoma. Tumor Biol 2005;26:195–200.
16. Kato Y, Sasagawa I, Kaneko M, Osawa M, Fujita N, and Tsuruo T: Aggrus: A diagnostic marker that distinguishes seminoma from embryonal carcinoma in testicular germ cell tumors. Oncogene 2004;23:8552–8556.
17. Kaneko MK, Oki H, Ogasawara S, Takagi M, and Kato Y: Anti-podoplanin monoclonal antibody LpMab-7 detects metastatic legions of osteosarcoma. Monoclon Antib Immunodiagn Immunother 2015;34:154–161.
18. Ariizumi T, Ogose A, Kawashima H, Hotta T, Li G, Xu Y, Umezu H, Sugai M, and Endo N: Expression of podoplanin in human bone and bone tumors: New marker of osteogenic and chondrogenic bone tumors. Pathol Int 2010;60:193–202.
19. Kunita A, Kashima TG, Ohazama A, Grigoriadis AE, and Fukayama M: Podoplanin is regulated by AP-1 and promotes platelet aggregation and cell migration in osteosarcoma. Am J Pathol 2011;179:1041–1049.
20. Ochoa-Alvarez JA, Krishnan H, Pastorino JG, Nevel E, Kephart D, Lee JJ, Retzbach EP, Shen Y, Fatahzadeh M, Baredes S, Kalyoussef E, Honma M, Adelson ME, Kaneko MK, Kato Y, Young MA, Deluca-Rapone L, Shienbaum AJ, Yin K, Jensen LD, and Goldberg GS: Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. Oncotarget 2015;6:9045–9060.
21. Mishima K, Kato Y, Kaneko MK, Nishikawa R, Hirose T, and Matsutani M: Increased expression of podoplanin in malignant astrocytic tumors as a novel molecular marker of malignant progression. Acta Neuropathol (Berl) 2006;111:483–488.
22. Mishima K, Kato Y, Kaneko MK, Nakazawa Y, Kunita A, Fujita N, Tsuruo T, Nishikawa R, Hirose T, and Matsutani M: Podoplanin expression in primary central nervous system germ cell tumors: A useful histological marker for the diagnosis of germinoma. Acta Neuropathol (Berl) 2006;111:563–568.
23. Kato Y, Vaidyanathan G, Kaneko MK, Mishima K, Srivastava N, Chandramohan V, Pegram C, Keir ST, Kuan CT, Bigner DD, and Zalutsky MR: Evaluation of anti-podoplanin rat monoclonal antibody NZ-1 for targeting malignant gliomas. Nucl Med Biol 2010;37:785–794.
24. Kato Y, and Kaneko MK: A cancer-specific monoclonal antibody recognizes the aberrantly glycosylated podoplanin. Sci Rep 2014;4:5924.

25. Kunita A, Kashima TG, Morishita Y, Fukayama M, Kato Y, Tsuruo T, and Fujita N: The platelet aggregation-inducing factor aggrus/podoplanin promotes pulmonary metastasis. Am J Pathol 2007;170:1337–1347.
26. Kaji C, Tsujimoto Y, Kato Kaneko M, Kato Y, and Sawa Y: Immunohistochemical examination of novel rat monoclonal antibodies against mouse and human podoplanin. Acta Histochem Cytochem 2012;45:227–237.
27. Oki H, Honma R, Ogasawara S, Fujii Y, Liu X, Takagi M, Kaneko MK, Kato Y: Development of sensitive monoclonal antibody PMab-2 against rat podoplanin. Monoclon Antib Immunodiagn Immunother 2015;34:396–403.
28. Honma R, Fujii Y, Ogasawara S, Oki H, Liu X, Nakamura T, Kaneko MK, Takagi M, and Kato Y: Establishment of a novel monoclonal antibody PMab-32 against rabbit podoplanin. Monoclon Antib Immunodiagn Immunother 2016; 35:41–47.
29. Honma R, Kaneko MK, Ogasawara S, Fujii Y, Konnai S, Takagi M, and Kato Y: Specific detection of dog podoplanin expressed in renal glomerulus by a novel monoclonal antibody PMab-38 in immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2016;35:212–216.
30. Yamada S, Itai S, Nakamura T, Yanaka M, Saidoh N, Chang YW, Handa S, Harada H, Kagawa Y, Ichii O, Konnai S, Kaneko MK, and Kato Y: PMab-52: Specific and sensitive monoclonal antibody against cat podoplanin for immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2017;36:224–230.
31. Honma R, Ogasawara S, Kaneko M, Fujii Y, Oki H, Nakamura T, Takagi M, Konnai S, and Kato Y: PMab-44 detects bovine podoplanin in immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2016;35:186–190.
32. Yamada S, Itai S, Nakamura T, Yanaka M, Kaneko MK, Kato Y: Detection of high CD44 expression in oral cancers using the novel monoclonal antibody, C44Mab-5. Biochem Biophys Rep 2018;14:64–68.
33. Itai S, Fujii Y, Nakamura T, Chang YW, Yanaka M, Saidoh N, Handa S, Suzuki H, Harada H, Yamada S, Kaneko MK, and Kato Y: Establishment of CMab-43, a sensitive and specific anti-CD133 monoclonal antibody, for immunohistochemistry. Monoclon Antib Immunodiagn Immunother 2017;36:231–235.
34. Yamada S, Itai S, Nakamura T, Yanaka M, Chang YW, Suzuki H, Kaneko MK, and Kato Y: Monoclonal antibody L1Mab-13 detected human PD-L1 in lung cancers. Monoclon Antib Immunodiagn Immunother 2018;37:110–115.
35. Oki H, Ogasawara S, Kaneko MK, Takagi M, Yamauchi M, and Kato Y: Characterization of monoclonal antibody LpMab-3 recognizing sialylated glycopeptide of podoplanin. Monoclon Antib Immunodiagn Immunother 2015;34:44–50.
36. Kaneko MK, Oki H, Hozumi Y, Liu X, Ogasawara S, Takagi M, Goto K, and Kato Y: Monoclonal antibody LpMab-9 recognizes O-glycosylated N-terminus of human podoplanin. Monoclon Antib Immunodiagn Immunother 2015;34:310–317.
37. Ogasawara S, Kaneko MK, and Kato Y: LpMab-19 recognizes sialylated O-glycan on Thr76 of human podoplanin. Monoclon Antib Immunodiagn Immunother 2016;35:245–253.
38. Kato Y, Kunita A, Fukayama M, Abe S, Nishioka Y, Uchida H, Tahara H, Yamada S, Yanaka M, Nakamura T, Saidoh N, Yoshida K, Fujii Y, Honma R, Takagi M, Ogasawara S, Murata T, and Kaneko MK: Antiglycopeptide mouse monoclonal antibody LpMab-21 exerts antitumor activity against human podoplanin through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Monoclon Antib Immunodiagn Immunother 2017;36:20–24.

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

Received: September 21, 2018
Accepted: December 20, 2018