MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume XX, Number XX, 2016

DOI: 10.1089/mab.2016.0022

Original Article

Specific Detection of Dog Podoplanin Expressed in Renal Glomerulus by a Novel Monoclonal Antibody PMab-38 in Immunohistochemistry

Ryusuke Honma,[1,2] Mika K. Kaneko,[1] Satoshi Ogasawara,[1] Yuki Fujii,[1] Satoru Konnai,[3] Michiaki Takagi,[2] and Yukinari Kato[1]

Podoplanin (PDPN) is expressed in several normal tissues including podocytes of renal glomerulus, lymphatic endothelial cells (LECs), and type I alveolar cells of lung. PDPN activates platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2) on platelet. Many monoclonal antibodies (mAbs) against human PDPN, mouse PDPN, rat PDPN, rabbit PDPN, and bovine PDPN have been established, antidog PDPN (dPDPN) mAbs have not been developed. Herein, we immunized mice with the recombinant proteins of dPDPN and developed anti-dPDPN mAbs. One of the clones, PMab-38, is useful for detecting podocytes in immunohistochemical analysis; in contrast, it did not react with LECs or type I alveolar cells. PMab-38 also detected dPDPN specifically in flow cytometry and Western blot analysis. PMab-38 is expected to be useful for investigating the function of dPDPN, which is expressed in podocytes.

Introduction

Podoplanin (PDPN), also known as Aggrus/T1α,[1–4] is a type I transmembrane sialoglycoprotein. Dog PDPN (dPDPN) was first reported as gp40.[5,6] PDPN is expressed in lymphatic endothelial cells (LECs), renal podocytes, pulmonary type I alveolar cells, osteocytes, and chondrocytes.[7,8] The expression of human PDPN (hPDPN) has been reported in many malignant tumors such as oral squamous cell carcinomas,[9] malignant brain tumors,[10–13] lung cancers,[14] esophageal cancers,[15] malignant mesotheliomas,[16,17] testicular tumors,[18] osteosarcomas,[8,19,20] and chondrosarcomas.[8] PDPN expression is also associated with malignant progression and cancer metastasis.[10,21,22]

PDPN activates platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2) on platelet.[21,23–25] The interaction between PDPN and CLEC-2 facilitates blood/lymphatic vessel separation.[26] PDPN is also expressed in human fetal rib and chondrocytes of the proliferative and hypertrophic regions of the growth plate.[27]

Herein, we immunized mice with the recombinant proteins of dPDPN and established anti-dPDPN monoclonal antibodies (mAbs).

Materials and Methods

Cell lines, dog tissues, and animals

Chinese hamster ovary (CHO)-K1 and P3U1 were purchased from the American Type Culture Collection (ATCC, Manassas, VA). CHO-K1, stable CHO transfectants, and P3U1 were cultured in RPMI 1640 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. Female BALB/c mice (4 weeks old) were purchased from CLEA Japan (Tokyo, Japan). Normal dog tissues were obtained from North lab (Hokkaido, Japan). Animals were housed under pathogen-free conditions. The Animal Care and Use Committee of Tohoku University approved the animal experiments described herein.

Hybridoma production

The dPDPN with N-terminal PA tag and C-terminal RAP tag-MAP tag (PA-dPDPN-RAP-MAP) was inserted into pCAG-zeo vector (Wako Pure Chemical Industries Ltd., Osaka, Japan). RAP tag consists of 12 amino acids (DMVNPGLEDRIE) and MAP tag consists of 12 amino acids (GDGMVPPGIEDK). CHO-K1 was transfected with pCAG-zeo/PA-dPDPN-RAP-MAP using Gene Pulser Xcell electroporation system (Bio-Rad Laboratories, Inc., Berkeley, CA). For the purification of PA-dPDPN-RAP-MAP from cell membrane, we used the PA tag system.[28,29] BALB/c mice were immunized by intraperitoneal (i.p.) injection of 100 μg of recombinant PA-dPDPN-RAP-MAP together with

[1]Department of Regional Innovation, Tohoku University Graduate School of Medicine, Sendai, Japan.
[2]Department of Orthopaedic Surgery, Yamagata University Faculty of Medicine, Yamagata, Japan.
[3]Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

Imject Alum (Thermo Fisher Scientific, Inc.). After several additional immunizations of 50 μg, a booster injection of 100 μg was given i.p. 2 days before spleen cells were harvested. The spleen cells were fused with P3U1 cells using PEG1500 (Roche Diagnostics, Indianapolis, IN). The hybridomas were grown in RPMI medium with hypoxanthine, aminopterin, and thymidine selection medium supplement (Thermo Fisher Scientific, Inc.).

The culture supernatants were screened using enzyme-linked immunosorbent assay (ELISA) for binding to purified recombinant PA-dPDPN-RAP-MAP. Recombinant proteins of PA-dPDPN-RAP-MAP were immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific, Inc.) at 1 μg/mL for 30 minutes. After blocking with 1% BSA in 0.05% Tween20/phosphate-buffered saline (PBS; Nacalai Tesque, Inc.), the plates were incubated with culture supernatant followed by 1:3000 diluted peroxidase-conjugated antimouse IgG (Dako; Agilent Technologies, Inc., Santa Clara, CA). The enzymatic reaction was conducted with a 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific, Inc.). The optical density was measured at 655 nm using an iMark microplate reader (Bio-Rad Laboratories, Inc.).

Immunohistochemical analyses

Four-micrometer-thick histologic sections were deparaffinized in xylene and rehydrated and were autoclaved in citrate buffer (pH 6.0; Dako) for 20 minutes. Sections were incubated with 10 μg/mL of primary mAbs for 1 hour at room temperature followed by treatment with Envision+ kit for 30 minutes (Dako). Color was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB; Dako) for 1 minute, and then the sections were counterstained with hematoxylin (Wako Pure Chemical Industries Ltd.).

Flow cytometry

Stable transfectant of CHO/PA-dPDPN-RAP-MAP was established using SH800 (Sony Corp., Tokyo, Japan). The other stable transfectants [CHO/PA-bovine PDPN (bovPDPN)-RAP-MAP, CHO/hPDPN-FLAG, CHO/mouse PDPN (mPDPN)-FLAG, CHO/rat PDPN (rPDPN)-His, and CHO/PA-rabbit PDPN (rabPDPN)] were previously established.[1,30–32] Cells were harvested by brief exposure to 0.25% trypsin/1 mM EDTA (Nacalai Tesque, Inc.). After washing with 0.1% BSA/PBS, the cells were treated with primary mAbs (1 μg/mL) for 30 minutes at 4°C followed by treatment with Oregon green-conjugated antimouse IgG (1:1000 diluted; Thermo Fisher Scientific, Inc.). Fluorescence data were collected using a Cell Analyzer EC800 (Sony Corp.).

Western blot analysis

Cell lysates (10 μg) of CHO/PA-dPDPN-RAP-MAP, CHO/bovPDPN-FLAG, CHO/hPDPN-FLAG, CHO/mPDPN-FLAG, CHO/rPDPN-His, CHO/PA-rabPDPN, and CHO-K1 were boiled in SDS sample buffer (Nacalai Tesque, Inc.). The proteins were electrophoresed on 5%–20% polyacrylamide gels (Wako Pure Chemical Industries Ltd.) and were transferred onto a polyvinylidene difluoride (PVDF) membrane (EMD Millipore Corp., Billerica, MA). After blocking with 4% skim milk (Nacalai Tesque, Inc.), the membrane was incubated with PMab-38 and anti-β-actin (clone AC-15; Sigma-Aldrich Corp., St. Louis, MO) and then with peroxidase-conjugated antimouse IgG (1:1000 diluted; Dako) and developed with the Pierce Western Blotting Substrate Plus (Thermo Fisher Scientific, Inc.) using a Sayaca-Imager (DRC Co. Ltd., Tokyo, Japan).

Results

Production of mAbs against dPDPN

We immunized mice with the recombinant proteins, which were purified from CHO/PA-dPDPN-RAP-MAP cells, and the ELISA screening was performed. Anti-PA tag mAb (clone: NZ-1), anti-MAP tag mAb (clone: PMab-1), and anti-RAP tag mAb (clone: PMab-2) detected the PA-dPDPN-RAP-MAP protein in Western blot analysis (data not shown). Among ELISA-positive wells, second screening was

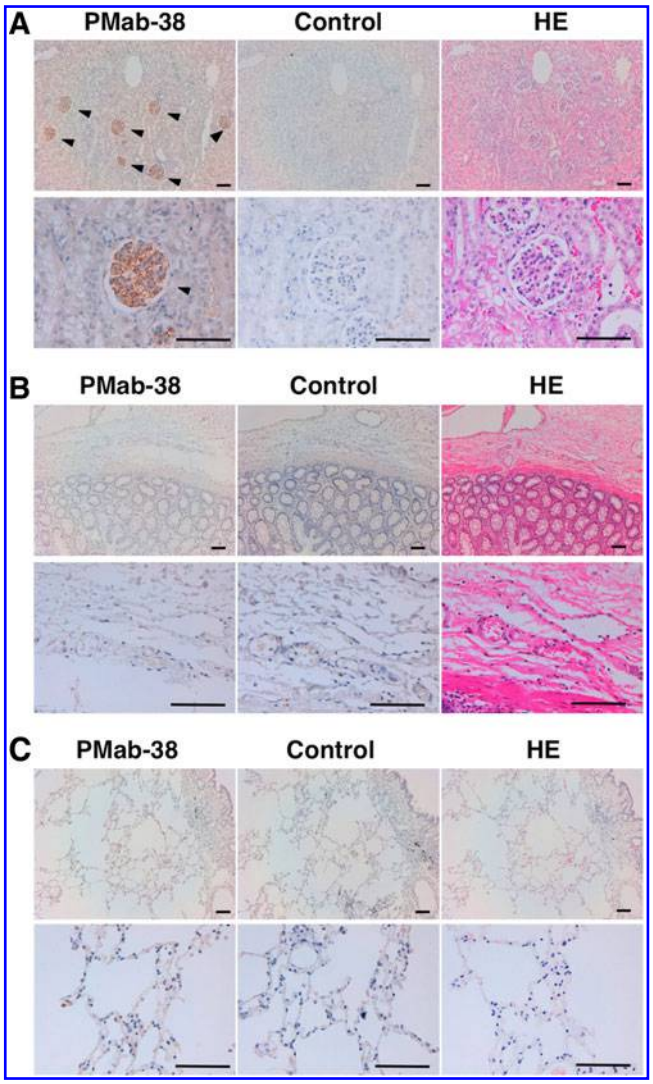

FIG. 1. Immunohistochemical analysis by PMab-38. Sections of dog kidney **(A)**, dog colon **(B)**, and dog lung **(C)** were autoclaved in citrate buffer (pH 6.0). After blocking, they were incubated with 10 μg/mL of PMab-38, followed by EnVision+ kit, and color was developed using DAB and counterstained with hematoxylin. PBS was used as negative control. HE staining was performed against serial sections. Arrow head, renal glomerulus. Scale bar: 100 μm. HE, hematoxylin and eosin.

performed by flow cytometry. Finally, positive wells were selected in immunohistochemical analysis. After limiting dilution, one of the clones, PMab-38 (IgG_1, kappa), was established. PMab-38 reacted with podocytes of renal glomerulus (Fig. 1A), whereas it did not react with other normal cells such as LECs (Fig. 1B) or type I alveolar cells of lung (Fig. 1C), in which PDPN expression has been reported in other species.[3,7] These data indicate that PMab-38 is useful for immunohistochemistry using paraffin-embedded tissues.

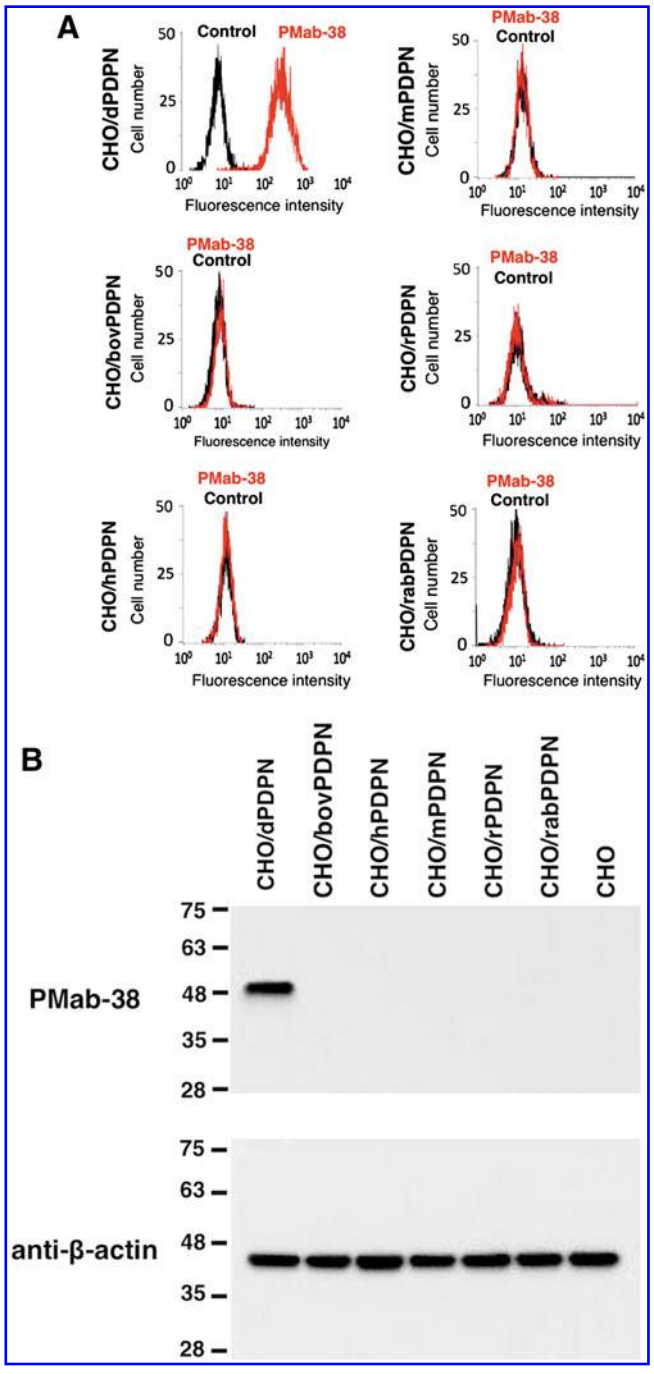

FIG. 2. Flow cytometric and Western blot analyses by PMab-38. **(A)** Cells were treated with PMab-38 followed by treatment with Oregon green-conjugated antimouse IgG. Black line: negative control. **(B)** Cell lysates (10 μg) were electrophoresed and transferred onto a PVDF membrane. After blocking, the membrane was incubated with 1 μg/mL of mAbs (PMab-38 or anti-β-actin), and then with peroxidase-conjugated antimouse IgG; the membrane was developed with Pierce Western Blotting Substrate Plus and detected using a Sayaca-Imager. mAbs, monoclonal antibodies; PVDF, polyvinylidene difluoride.

Specificity of PMab-38 against dPDPN in flow cytometry and Western blot

PMab-38 reacted with CHO/PA-dPDPN-RAP-MAP, not with CHO/PA-bovPDPN-RAP-MAP, CHO/hPDPN-FLAG, CHO/mPDPN-FLAG, CHO/rPDPN-His, CHO/PA-rabPDPN, and CHO-K1 cells in flow cytometry (Fig. 2A). In Western blot analysis, PMab-38 recognized only dPDPN of CHO/PA-dPDPN-RAP-MAP, and did not react with PDPNs of CHO/bovPDPN-FLAG, CHO/hPDPN-FLAG, CHO/mPDPN-FLAG, CHO/rPDPN-His, CHO/PA-rabPDPN, or CHO-K1 cells (Fig. 2B). These results indicate that PMab-38 detects dPDPN specifically.

Discussion

Although many mAbs and polyclonal Abs against PDPN have been produced, anti-dPDPN mAbs have not been developed. Our previous reports revealed that PMab-44 (anti-bovPDPN mAb; mouse IgG_1),[32] LpMab-12 (anti-hPDPN mAb; mouse IgG_1),[33] PMab-1 (anti-mPDPN mAb; rat IgG_{2a}),[34] PMab-2 (anti-rPDPN mAb; mouse IgG_1),[30] and PMab-32 (anti-rabPDPN mAb; mouse IgG_1)[31,35] specifically react with CHO/bovPDPN, CHO/hPDPN, CHO/mPDPN, CHO/rPDPN, and CHO/rabPDPN, respectively. Many other anti-hPDPN mAbs also detect hPDPN specifically.[12,13,17,19,36–47] Herein, we immunized mice with the recombinant proteins of dPDPN, and developed several anti-dPDPN mAbs including PMab-38. When PMab-38 was incubated for 18 hours at 4°C in immunohistochemistry, the stronger staining was observed in renal glomerulus, and Bowman's capsule was also stained by PMab-38, whereas LECs or type I alveolar cells were not stained (data not shown). These data demonstrate that PMab-38 is useful for immunohistochemistry using paraffin-embedded tissues, but the reaction of PMab-38 is limited to podocytes of renal glomerulus.

Taken together, PMab-38 could be useful to uncover the pathophysiological function of dPDPN in podocytes of renal glomerulus. In the future, we should determine the epitope of PMab-38 and clarify the reason for the specificity of PMab-38 against podocytes of renal glomerulus in immunohistochemistry.

Acknowledgments

We thank Takuro Nakamura, Noriko Saidoh, and Kanae Yoshida for their excellent technical assistance. This work was supported in part by the Regional Innovation Strategy Support Program from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (Y.K.), by JSPS KAKENHI Grant Number 26440019 (M.K.K.) and Grant Number 25462242 and 16K10748 (Y.K.), by Takeda Science Foundation (S.O.), by the Platform for Drug Discovery, Informatics, and Structural Life Science (PDIS) from Japan Agency for Medical Research and Development, AMED (Y.K.), and by the Basic Science and Platform Technology Program for Innovative Biological Medicine from AMED (Y.K.). This work was performed, in part, under

the Cooperative Research Program of Institute for Protein Research, Osaka University, CR-15-05 and CR-16-05.

Author Disclosure Statement

No competing financial interests exist.

References

1. 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.
2. 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.
3. 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.
4. 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.
5. Zimmer G, Klenk HD, and Herrler G: Identification of a 40-kDa cell surface sialoglycoprotein with the characteristics of a major influenza C virus receptor in a Madin-Darby canine kidney cell line. J Biol Chem 1995;270: 17815–17822.
6. Zimmer G, Oeffner F, Von Messling V, Tschernig T, Groness HJ, Klenk HD, and Herrler G: Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium. Biochem J 1999;341:277–284.
7. 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.
8. 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.
9. 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.
10. 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 2006;111:483–488.
11. 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 2006;111:563–568.
12. 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.
13. Kato Y, and Kaneko MK: A cancer-specific monoclonal antibody recognizes the aberrantly glycosylated podoplanin. Sci Rep 2014;4:5924.
14. 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.
15. 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.
16. Kimura N, and Kimura I: Podoplanin as a marker for mesothelioma. Pathol Int 2005;55:83–86.
17. 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.
18. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. 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.
28. Fujii Y, Kaneko M, Neyazaki M, Nogi T, Kato Y, and Takagi J: PA tag: A versatile protein tagging system using a super high affinity antibody against a dodecapeptide derived from human podoplanin. Protein Expr Purif 2014;95: 240–247.
29. Fujii Y, Matsunaga Y, Arimori T, Kitago Y, Ogasawara S, Kaneko MK, Kato Y, and Takagi J: Tailored placement of a turn-forming PA tag into the structured domain of a protein to probe its conformational state. J Cell Sci 2016;129: 1512–1522.
30. Oki H, Honma R, Ogasawara S, Fujii Y, Liu X, Takagi M, Kaneko MK, and Kato Y: Development of sensitive monoclonal antibody PMab-2 against rat podoplanin. Monoclon Antib Immunodiagn Immunother 2015;34:396–403.
31. 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.
32. 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;DOI: 10.1089/mab.2016.0016.
33. Kato Y, Ogasawara S, Oki H, Goichberg P, Honma R, Fujii Y, and Kaneko MK: LpMab-12 established by CasMab technology specifically detects sialylated O-Glycan on Thr52 of platelet aggregation-stimulating domain of human podoplanin. PLoS One 2016;11:e0152912.
34. 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.
35. Honma R, Fujii Y, Ogasawara S, Oki H, Konnai S, Kagawa Y, Takagi M, Kaneko MK, and Kato Y: Critical epitope of anti-rabbit podoplanin monoclonal antibodies for immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2016;35:65–72.
36. Kato Y, Ogasawara S, Oki H, Honma R, Takagi M, Fujii Y, Nakamura T, Saidoh N, Kanno H, Umetsu M, Kamata S, Kubo H, Yamada M, Sawa Y, Morita K, Harada H, Suzuki H, and Kaneko MK: Novel monoclonal antibody LpMab-17 developed by CasMab technology distinguishes human podoplanin from monkey podoplanin. Monoclon Antib Immunodiagn Immunother 2016;35:109–116.
37. 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.
38. Oki H, Kaneko MK, Ogasawara S, Tsujimoto Y, Liu X, Sugawara M, Takakubo Y, Takagi M, and Kato Y: Characterization of a monoclonal antibody LpMab-7 recognizing non-PLAG domain of podoplanin. Monoclon Antib Immunodiagn Immunother 2015;34:174–180.
39. 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.
40. Ogasawara S, Kaneko MK, Honma R, Oki H, Fujii Y, Takagi M, Suzuki H, and Kato Y: Establishment of mouse monoclonal antibody LpMab-13 against human podoplanin. Monoclon Antib Immunodiagn Immunother 2016;35:155–162.
41. Chandramohan V, Bao X, Kato Kaneko M, Kato Y, Keir ST, Szafranski SE, Kuan CT, Pastan IH, and Bigner DD: Recombinant anti-podoplanin (NZ-1) immunotoxin for the treatment of malignant brain tumors. Int J Cancer 2013; 132:2339–2348.
42. Ogasawara S, Kaneko MK, Price JE, and Kato Y: Characterization of anti-podoplanin monoclonal antibodies: Critical epitopes for neutralizing the interaction between podoplanin and CLEC-2. Hybridoma 2008;27:259–267.
43. Sekiguchi T, Takemoto A, Takagi S, Takatori K, Sato S, Takami M, and Fujita N: Targeting a novel domain in podoplanin for inhibiting platelet-mediated tumor metastasis. Oncotarget 2016;7:3934–3946.
44. Takagi S, Sato S, Oh-hara T, Takami M, Koike S, Mishima Y, Hatake K, Fujita N: Platelets promote tumor growth and metastasis via direct interaction between Aggrus/podoplanin and CLEC-2. PLoS One 2013;8:e73609.
45. Nakazawa Y, Takagi S, Sato S, Oh-hara T, Koike S, Takami M, Arai H, and Fujita N: Prevention of hematogenous metastasis by neutralizing mice and its chimeric anti-Aggrus/podoplanin antibodies. Cancer Sci 2011;102:2051–2057.
46. Kato Y, Kunita A, Abe S, Ogasawara S, Fujii Y, Oki H, Fukayama M, Nishioka Y, and Kaneko MK: The chimeric antibody chLpMab-7 targeting human podoplanin suppresses pulmonary metastasis via ADCC and CDC rather than via its neutralizing activity. Oncotarget 2015;6:36003–36018.
47. Kono T, Shimoda M, Takahashi M, Matsumoto K, Yoshimoto T, Mizutani M, Tabata C, Okoshi K, Wada H, and Kubo H: Immunohistochemical detection of the lymphatic marker podoplanin in diverse types of human cancer cells using a novel antibody. Int J Oncol 2007;31:501–508.

Address correspondence to:
Prof. Yukinari Kato
Department of Regional Innovation
Tohoku University Graduate School of Medicine
2-1 Seiryo-machi, Aoba-ku
Sendai 980-8575
Japan

E-mail: yukinari-k@bea.hi-ho.ne.jp;
yukinarikato@med.tohoku.ac.jp

Received: May 12, 2016
Accepted: June 3, 2016