MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 38, Number 2, 2019
© Mary Ann Liebert, Inc.
DOI: 10.1089/mab.2019.0001

Epitope Mapping of the Antihorse Podoplanin Monoclonal Antibody PMab-202

Mika K. Kaneko,[1] Yoshikazu Furusawa,[1–3] Masato Sano,[1] Shunsuke Itai,[1,4] Junko Takei,[1,4] Hiroyuki Harada,[4] Masato Fukui,[3] Shinji Yamada,[1] and Yukinari Kato[1,2]

Horse podoplanin (horPDPN), a type I transmembrane sialoglycoprotein, is expressed on the podocytes of the kidneys, alveolar type I cells of the lungs, and lymphatic endothelial cells. PDPN is a platelet aggregation-inducing factor, and it primarily possesses three platelet aggregation-stimulating (PLAG) domains, that is, PLAG1, PLAG2, and PLAG3, at the N-terminus and several PLAG-like domains. In a previous study, we reported on a mouse anti-horPDPN monoclonal antibody (mAb) clone, PMab-202. Although the effectiveness of PMab-202 in flow cytometry and Western blotting is known, its exact binding epitope remains unknown to date. In this study, enzyme-linked immunosorbent assay and flow cytometry were used to identify the epitope of PMab-202. We found that the critical epitopes of PMab-202 include Lys64, Thr66, and Phe70 of horPDPN. We believe that our findings can be applied in the production of more functional anti-horPDPN mAbs.

Keywords: podoplanin, PDPN, PMab-202, epitope mapping

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.

Introduction

Podoplanin (PDPN) induces platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2).[1–8] PDPN is a type I transmembrane sialoglycoprotein expressed in normal tissues, including renal corpuscles, alveolar type I cells of the lungs, and lymphatic endothelial cells.[3,9] The interaction between the PDPN of lymphatic endothelial cells and CLEC-2 of platelets facilitates embryonic blood/lymphatic vessel separation.[10] Expression of human PDPN, a protein associated with malignant progression and cancer metastasis,[5,11,12] has been reported in several malignant tumors, such as brain tumors,[11,13–15] mesotheliomas,[16,17] oral squamous cell carcinomas,[18] esophageal cancers,[19] lung cancers,[20] osteosarcomas,[21–23] and testicular tumors.[24]

In a previous study, we developed monoclonal antibodies (mAbs) not only against human,[25] mouse,[25] and rat[26] PDPNs but also against rabbit,[27] dog,[28] cat,[29] bovine,[30] pig,[31] and horse[32–34] PDPNs (horPDPNs). PDPN primarily possesses three platelet aggregation-stimulating (PLAG) domains: PLAG1, PLAG2, and PLAG3; these domains are present at the N-terminus of PDPN.[2] One PLAG-like domain (PLD), important for PDPN–CLEC-2 interaction, has been reported to be present in the middle of PDPN.[35] Almost all mAbs against PDPNs have been reported to react with the PLAG domains or PLDs.[35,36]

In this study, we determined the epitope responsible for the binding of PMab-202 to horPDPN using enzyme-linked immunosorbent assay (ELISA) and flow cytometry.

Materials and Methods

Cell line

Chinese hamster ovary (CHO)-K1 was obtained from American Type Culture Collection (ATCC, Manassas, VA). The horse kidney cell line FHK-Tcl3.1 was prepared at Yamaguchi University.[37] horPDPN bearing an N-terminal PA16 tag (PA16-horPDPN) was inserted into a pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).[33] The PA16 tag comprised 16 amino acids (GLEGGVAMPGAEDDVV).[38] Furthermore, the CHO-K1 cells were transfected with pCAG-Ble/PA16-horPDPN using Lipofectamine LTX with Plus Reagent (Thermo Fisher Scientific, Inc., Waltham, MA). Stable transfectants were selected by limiting dilution and cultivating in a medium containing 0.5 mg/mL Zeocin (InvivoGen, San Diego, CA). The CHO/horPDPN cells were cultured in Roswell Park

[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, Tokyo, Japan.

Table 1. Determination of PMab-202 Epitope by Enzyme-Linked Immunosorbent Assay

Peptide	*Sequence*	*PMab-202*
pp23-42	ASTLGPEDNIMTPGVEDGMV	–
pp33-52	MTPGVEDGMVTPGGSEDSES	–
pp43-62	TPGGSEDSESTGSPALVPRS	–
pp53-72	TGSPALVPRSTKSTGGDFED	+++
pp63-82	TKSTGGDFEDRSTLGNTVHT	+++
pp73-92	RSTLGNTVHTPGESQSTRTP	–
pp83-102	PGESQSTRTPSVLTGHPTEK	–
pp93-112	SVLTGHPTEKTDGNTKATVE	–
pp103-118	TDGNTKATVEKDGLST	–

+++, OD655 ≧ 0.6; –, OD655 < 0.1.

Table 2. Determination of PMab-202 Epitope by Enzyme-Linked Immunosorbent Assay

Mutation	*Sequence*	*PMab-202*
T53A	AGSPALVPRSTKSTGGDFED	+++
G54A	TASPALVPRSTKSTGGDFED	+++
S55A	TGAPALVPRSTKSTGGDFED	+++
P56A	TGSAALVPRSTKSTGGDFED	+++
A57G	TGSPGLVPRSTKSTGGDFED	+++
L58A	TGSPAAVPRSTKSTGGDFED	+++
V59A	TGSPALAPRSTKSTGGDFED	+++
P60A	TGSPALVARSTKSTGGDFED	+++
R61A	TGSPALVPASTKSTGGDFED	+++
S62A	TGSPALVPRATKSTGGDFED	+++
T63A	TGSPALVPRSAKSTGGDFED	+++
K64A	TGSPALVPRSTASTGGDFED	+
S65A	TGSPALVPRSTKATGGDFED	+++
T66A	TGSPALVPRSTKSAGGDFED	+
G67A	TGSPALVPRSTKSTAGDFED	+++
G68A	TGSPALVPRSTKSTGADFED	+++
D69A	TGSPALVPRSTKSTGGAFED	+++
F70A	TGSPALVPRSTKSTGGDAED	–
E71A	TGSPALVPRSTKSTGGDFAD	+++
D72A	TGSPALVPRSTKSTGGDFEA	+++

+++, OD655 ≧ 0.6; +, 0.1 ≦ OD655 < 0.4; –, OD655 < 0.1.

Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), whereas FHK-Tcl3.1 was cultured in Dulbecco's modified Eagle's medium (DMEM; Nacalai Tesque, Inc.).[33] RPMI 1640 and DMEM were supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 units/mL penicillin, 100 μg/mL streptomycin, and 25 μg/mL amphotericin B (Nacalai Tesque, Inc.). The cells were grown at 37°C in a humidified environment under a 5% CO_2 atmosphere.

Enzyme-linked immunosorbent assay

The horPDPN peptides synthesized using PEPScreen (Sigma-Aldrich Corp., St. Louis, MO) were immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific, Inc.) at concentrations of 1 μg/mL for 30 minutes at 37°C. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), the plates were incubated with 1 μg/mL purified PMab-202, followed by 1:2000 dilution of peroxidase-conjugated antimouse IgG (Agilent Technologies, Inc., Santa Clara, CA). The enzymatic reaction was performed using 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific, Inc.). Optical density was measured at 655 nm using an iMark microplate reader (Bio-Rad Laboratories, Inc., Berkeley, CA). These reactions were performed at 37°C using a total sample volume of 50–100 μL.

Flow cytometry

CHO/horPDPN or FHK-Tcl3.1 cells were harvested after a brief exposure to 0.25% trypsin/1 mM EDTA (Nacalai Tesque, Inc.), and were washed with 0.1% bovine serum albumin/phosphate-buffered saline. CHO/horPDPN cells were treated with 0.1 μg/mL PMab-202 or 0.1 μg/mL PMab-202 plus 50 μg/mL peptides for 30 minutes at 4°C. FHK-Tcl3.1 cells were treated with 1 μg/mL PMab-202 or 1 μg/mL PMab-202 plus 10 μg/mL peptides for 30 minutes at 4°C. Thereafter, the cells were treated with Alexa Fluor 488-conjugated antimouse IgG (1:2000; Cell Signaling Technology, Inc., Danvers, MA). Fluorescence data were collected using SA3800 Cell Analyzers (Sony Corp., Tokyo, Japan).

Results and Discussion

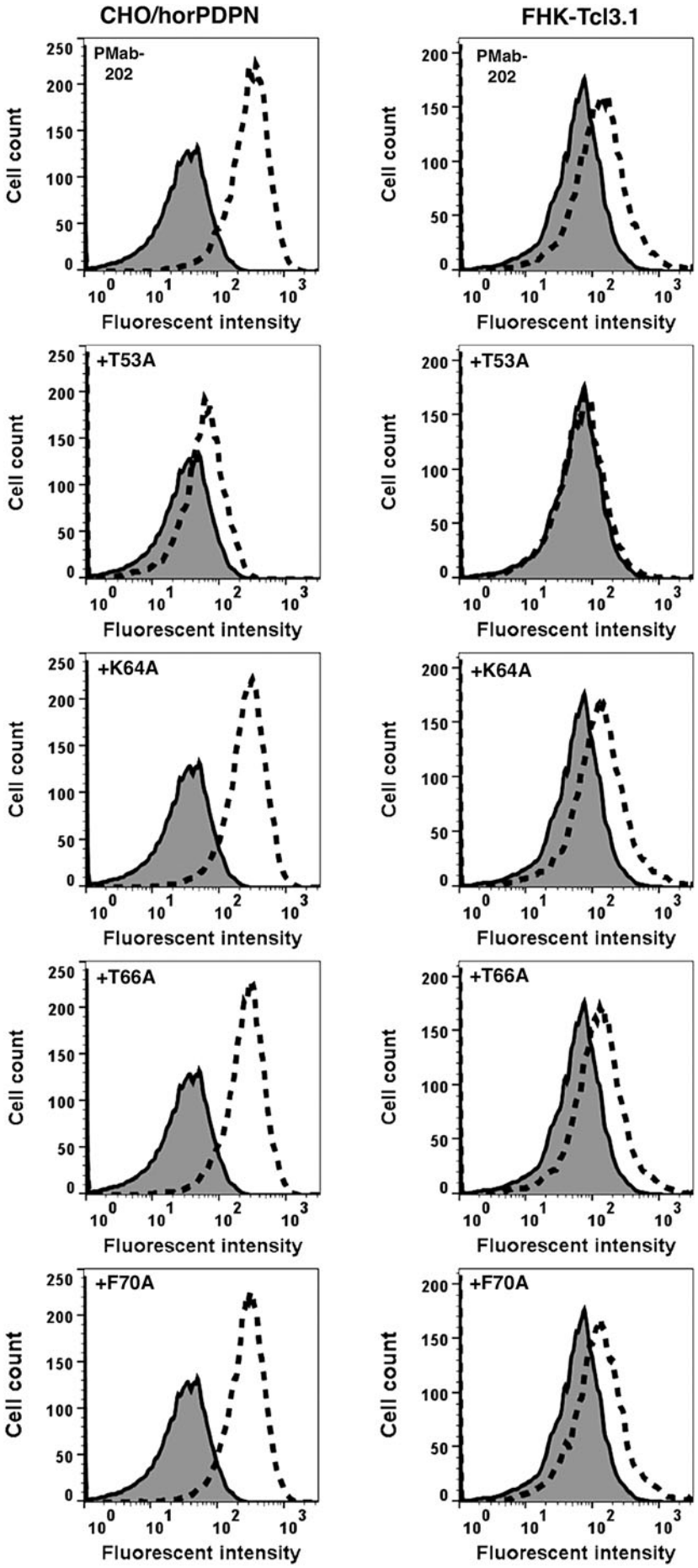
In a previous study, we immunized mice with a synthesized peptide (horPP6279) corresponding to the amino acids 62–79 of horPDPN.[33] ELISA screening indicated positive signals in 21 of 960 (2.2%) wells. Among these 21 wells, 6 (29%) tested positive against CHO/horPDPN in flow cytometry. One clone, that is, PMab-202 (IgG_1, kappa), among the six positive clones was established by limiting dilution. PMab-202 could detect endogenous horPDPN expressed in FHK-Tcl3.1, a horse kidney cell line, in flow cytometry and Western blotting. However, PMab-202 was not advantageous for immunohistochemical analysis.

We first synthesized a series of peptides of horPDPN (Table 1). Using ELISA, PMab-202 detected 53–72 and 63–82 corresponding to the amino acids 53–72 and 63–82 of horPDPN, respectively. Next, we synthesized the point mutants of 53–72 peptides (Table 2). Using ELISA, PMab-202 detected the following antigens: T53A, G54A, S55A, P56A, A57G, L58A, V59A, P60A, R61A, S62A, T63A, S65A, G67A, G68A, D69A, E71A, and D72A. However, F70A was not recognized, and it weakly reacted with K64A and T66A, indicating that Lys64, Thr66, and Phe70 are the critical epitopes of PMab-202.

FIG. 1. Flow cytometry using PMab-202 and point mutants of horPDPN. CHO/horPDPN cells were treated with 0.1 μg/mL PMab-202 or 0.1 μg/mL PMab-202 plus 50 μg/mL peptides for 30 minutes at 4°C. FHK-Tcl3.1 cells were treated with 1 μg/mL PMab-202 or 1 μg/mL PMab-202 plus 10 μg/mL peptides for 30 minutes at 4°C. Thereafter, the cells were treated with Alexa Fluor 488-conjugated antimouse IgG. Solid line with gray shade, control (second Ab only); dotted line, PMab-202 or PMab-202 plus peptides. CHO, Chinese hamster ovary; horPDPN, horse podoplanin.

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.



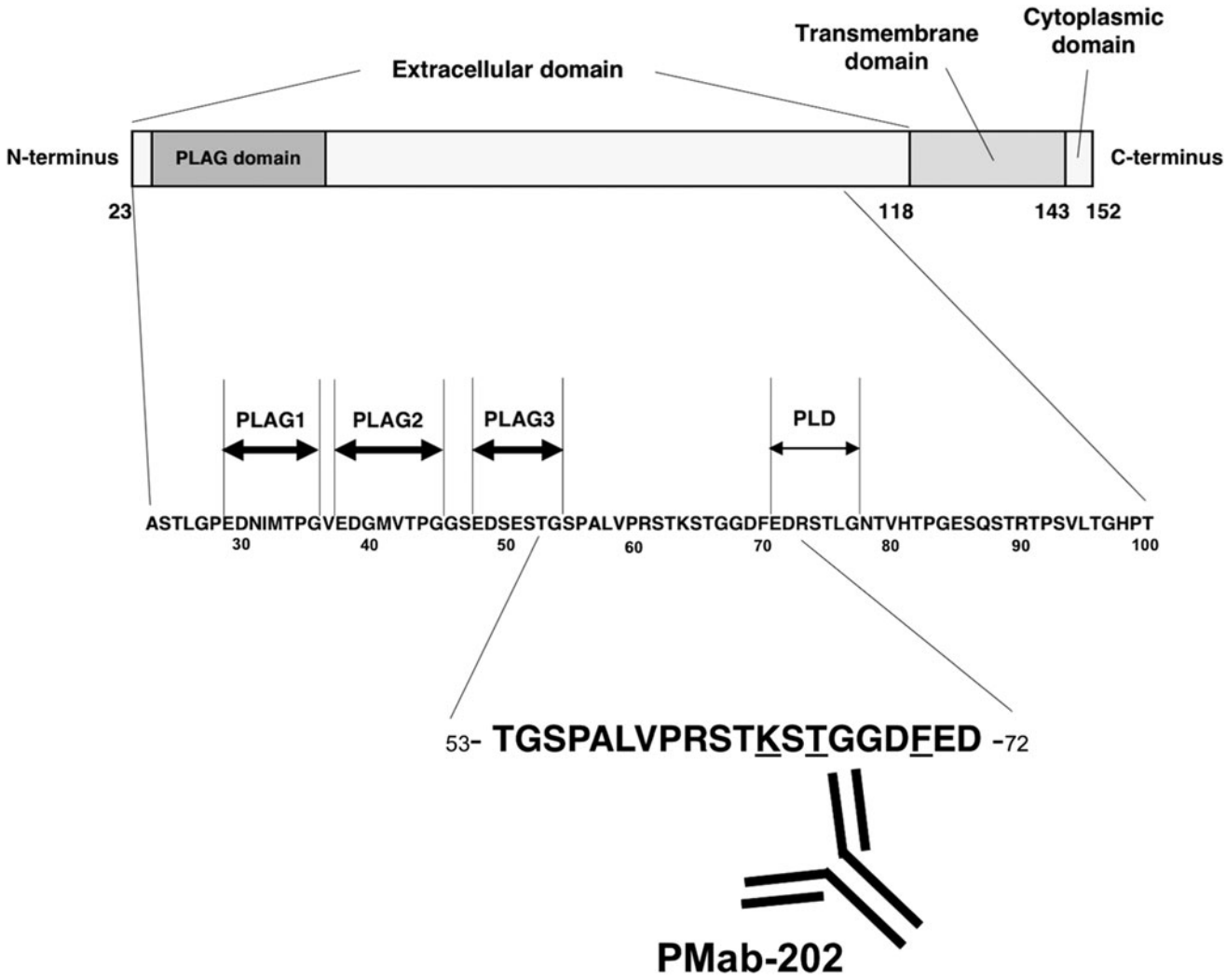


FIG. 2. Schematic illustration of the epitope recognized by PMab-202. Underlined amino acids are the critical epitope PMab-202. PLAG, platelet aggregation-stimulating; PLD, PLAG-like domain.

Next, we performed a blocking assay using flow cytometry. We found that PMab-202 reacted with the CHO/horPDPN cell line (Fig. 1). This reaction was partially neutralized by T53A. However, K64A, T66A, and F70A did not block the reaction of PMab-202 with CHO/horPDPN. Similarly, PMab-202 reacted with the FHK-Tcl3.1 cell line (Fig. 1). Notably, this reaction was completely neutralized by T53A. In contrast, K64A, T66A, and F70A did not block the reaction of PMab-202 with FHK-Tcl3.1, thereby confirming that Lys64, Thr66, and Phe70 of horPDPN are critical for PMab-202 detection. As shown in Figure 2, Lys64, Thr66, and Phe70 are included between PLAG3 and PLD.

Taken together, the study findings indicate that the critical epitopes of PMab-202 are Lys64, Thr66, and Phe70 of horPDPN. We believe that these findings will be beneficial in the production of more functional anti-horPDPN mAbs.

Acknowledgments

We thank Takuro Nakamura, Miyuki Yanaka, Saori Handa, Kayo Hisamatsu, and Yoshimi Nakamura for excellent technical assistance. FHK-Tcl3.1 was kindly provided by Ken Maeda (Yamaguchi University). This research was supported, in part, by AMED under Grant Nos.: JP18am0101078 (Y.K.), JP18am0301010 (Y.K.), and JP18ae0101028 (Y.K.), and by JSPS KAKENHI Grant No. 17K07299 (M.K.K.) and Grant No. 16K10748 (Y.K.).

Author Disclosure Statement

Y.K. received research funding from ZENOAQ Resource Co., Ltd. The other authors have no conflict of interest.

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.

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.

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. 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.
6. 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.
7. 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.
8. 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.
9. 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.
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. 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.
12. 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.
13. 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.
14. 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.
15. Kato Y, and Kaneko MK: A cancer-specific monoclonal antibody recognizes the aberrantly glycosylated podoplanin. Sci Rep 2014;4:5924.
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. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
31. Kato Y, Yamada S, Furusawa Y, Itai S, Nakamura T, Yanaka M, Sano M, Harada H, Fukui M, and Kaneko MK: PMab-213: A monoclonal antibody for immunohistochemical analysis against pig podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:18–24.
32. Kato Y, Yamada S, Itai S, Kobayashi A, Konnai S, and Kaneko MK: Anti-horse podoplanin monoclonal antibody PMab-219 is useful for detecting lymphatic endothelial cells by immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2018;37:272–274.
33. Furusawa Y, Yamada S, Itai S, Sano M, Nakamura T, Yanaka M, Handa S, Mizuno T, Maeda K, Fukui M, Harada H, Kaneko MK, and Kato Y: Establishment of monoclonal antibody PMab-202 against horse podoplanin. Monoclon Antib Immunodiagn Immunother 2018;37:233–237.
34. Furusawa Y, Yamada S, Itai S, Nakamura T, Yanaka M, Sano M, Harada H, Fukui M, Kaneko MK, and Kato Y: PMab-219: A monoclonal antibody for the immunohistochemical analysis of horse podoplanin. Biochem Biophys Rep 2017;18:100616.
35. Sekiguchi T, Takemoto A, Takagi S, Takatori K, Sato S, Takami M, and Fujita N: Targeting a novel domain in podoplanin for inhibiting plateletmediated tumor metastasis. Oncotarget 2015;7:3934–3946.
36. Kaneko MK, Nakamura T, Honma R, Ogasawara S, Fujii Y, Abe S, Takagi M, Harada H, Suzuki H, Nishioka Y, and Kato Y: Development and characterization of anti-glycopeptide monoclonal antibodies against human podoplanin, using glycan-deficient cell lines generated by CRISPR/Cas9 and TALEN. Cancer Med 2017;6:382–396.
37. Andoh K, Kai K, Matsumura T, and Maeda K: Further development of an equine cell line that can be propagated over 100 times. J Equine Sci 2009;20:11–14.
38. Yamada S, Itai S, Nakamura T, Yanaka M, Kaneko MK, and Kato Y: Detection of high CD44 expression in oral cancers using the novel monoclonal antibody, C44Mab-5. Biochem Biophys Rep 2018;14:64–68.

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

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

Received: January 16, 2019
Accepted: March 4, 2019

Downloaded by Yukinari Kato from www.liebertpub.com at 04/22/19. For personal use only.