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Epitope Mapping of Antipig Podoplanin Monoclonal Antibody PMab-213

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Podoplanin (PDPN), a type I transmembrane sialoglycoprotein, is expressed on lymphatic endothelial cells, podocytes of the kidneys, and type I alveolar cells of the lungs. PDPN, a platelet aggregation-inducing factor, comprises three platelet aggregation-stimulating (PLAG) domains (PLAG1, PLAG2, and PLAG3) in the N-terminus and PLAG-like domains in the middle of the PDPN protein. We have previously reported a mouse antipig PDPN (pPDPN) monoclonal antibody (mAb) clone, PMab-213, which was developed using the Cell-Based Immunization and Screening (CBIS) method. PMab-213 is very useful in flow cytometry, Western blotting, and immunohistochemical analyses; however, the binding epitope of PMab-213, which was developed by CBIS method, remains to be elucidated. Therefore, this study aimed to investigate the epitope of PMab-213 using enzyme-linked immunosorbent assay, flow cytometry, and immunohistochemical analyses. The results revealed that the critical epitopes of PMab-213 are Pro53, Arg54, Arg56, and Tyr60 of pPDPN.

Keywords: podoplanin, PDPN, PMab-213, epitope mapping

Introduction

PODOPLANIN (PDPN) IS A TYPE I transmembrane sialoglycoprotein that is expressed in normal tissues, including type I lung alveolar cells, renal corpuscles, and lymphatic endothelial cells.[1,2] PDPN binds to C-type lectin-like receptor-2 (CLEC-2) and thus induces platelet aggregation.[1,3–9] The interaction between PDPN on lymphatic endothelial cells and CLEC-2 on platelets facilitates the separation of embryonic blood/lymphatic vessels.[10] Studies have reported the expression of human PDPN in several malignant tumors, such as oral squamous cell carcinomas,[11] malignant brain tumors,[12–15] esophageal cancers,[16] malignant mesotheliomas,[17,18] lung cancers,[19] testicular tumors,[20] and osteosarcomas.[21–23] PDPN expression has also been associated with malignant progression and cancer metastasis.[6,12,24] Although pathophysiological studies of alveolar cells of the lungs, podocytes of the kidneys, or lymphatic endothelial cells of the colon and skin using antipig PDPN monoclonal antibodies (mAbs) are important in many disorders, mAbs against pPDPN remain to be developed.

In our previous study, we used the Cell-Based Immunization and Screening (CBIS) method to develop specific and sensitive mAbs against the pig PDPN (pPDPN) of 159 amino acids (aas) to facilitate the immunohistochemical analysis of paraffin-embedded tissue sections.[25] Flow cytometry revealed that PMab-213 (mouse IgG_{2b}, kappa), an established clone, recognized Chinese hamster ovary (CHO)/pPDPN but did not react with CHO-K1. PMab-213 also reacted with the PK-15 pig kidney cell line, indicating that PMab-213 recognizes endogenous pPDPN. Western blotting performed demonstrated the ability of PMab-213 to detect pPDPN as 40- and 25-kDa bands in CHO/pPDPN cells. Moreover, the K_D of PMab-213 for CHO/pPDPN was determined to be 2.1×10^{-9} M, indicating its high affinity for CHO/pPDPN. Immunohistochemical analyses revealed that PMab-213 strongly stained pulmonary type I alveolar cells, podocytes, and Bowman's capsules in the kidney, and lymphatic endothelial cells obtained from microminipigs. This study aimed to determine the binding epitope of PMab-213 to pPDPN using enzyme-linked immunosorbent assay (ELISA), flow cytometry, and immunohistochemical analyses.

Materials and Methods

Cell line

CHO-K1 was 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 (JCRB; Osaka, Japan). pPDPN (accession no.: XM_005665017) bearing an N-terminal PA16 tag (PA16-pPDPN) was previously inserted into a pCAG-Ble vector (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The PA16 tag comprised 16 aas (GLEGGVAMPGAEDDVV).[25–27] pCAG-Ble/PA16-pPDPN

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was transfected into CHO-K1 cells using Lipofectamine LTX with Plus Reagent (Thermo Fisher Scientific, Inc., Waltham, MA). Stable transfectants were selected by limiting the dilution and cultivating them in a medium containing 0.5 mg/mL zeocin (InvivoGen, San Diego, CA). CHO/pPDPN cells were cultured in RPMI 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan), whereas PK-15 cells were cultured in DMEM medium (Nacalai Tesque, Inc.). All media were supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 μg/mL amphotericin B (Nacalai Tesque, Inc.) and stored at 37°C in a humidified chamber under 5% CO_2 and 95% air atmosphere.

Enzyme-linked immunosorbent assay

Synthesized pPDPN peptides using PEPScreen (Sigma-Aldrich Corp., St. Louis, MO) were immobilized on Nunc Maxisorp 96-well immunoplates (Thermo Fisher Scientific, Inc.) at 1 μg/mL for 30 min at 37°C. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), the plates were incubated with purified PMab-213 (1 μg/mL), followed by a peroxidase-conjugated antimouse IgG (Agilent Technologies, Inc., Santa Clara, CA) dilution of 1:2000. Enzymatic reactions were 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

Cells were harvested after a brief exposure to 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (Nacalai Tesque, Inc.). The cells were then washed with 0.1% bovine serum albumin/phosphate-buffered saline (PBS) and treated with PMab-213 (1 μg/mL) or PMab-213 (1 μg/mL) + peptides (10 μg/mL) for 30 min at 4°C. The cells were then treated with Alexa Fluor 488-conjugated antimouse IgG (1:2000; Cell Signaling Technology, Inc., Danvers, MA). Fluorescence data were collected using the SA3800 Cell Analyzers (Sony Corp., Tokyo, Japan).

Immunohistochemical analyses

Microminipig (Fuji Micra, Inc., Shizuoka, Japan) tissues were collected on autopsy at the Gifu University and then fixed in 4% paraformaldehyde. The tissues were processed to produce 4-μm paraffin-embedded tissue sections, which were directly autoclaved in citrate buffer (pH 6.0; Nichirei Biosciences, Inc., Tokyo, Japan) for 20 min. These tissue sections were then blocked with the SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc.), incubated with PMab-213 (1 μg/mL) or PMab-213 (1 μg/mL) + peptides (1 μg/mL) for 1 hour at room temperature, and treated using an Envision + Kit (Agilent Technologies, Inc.) for 30 min. Color was developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Agilent Technologies, Inc.) for 2 min, and counterstaining was performed using hematoxylin (FUJIFILM Wako Pure Chemical Corporation).

Table 1. Determination of PMab-213 Epitope by Enzyme-Linked Immunosorbent Assay

Peptide	*Sequence*	*PMab-213*
pp23-42	ASTVLPEDGVTPGVEGSSKA	–
pp33-52	TPGVEGSSKASPGVEDYTVT	–
pp43-62	SPGVEDYTVTPRTREEPYAT	+++
pp53-72	PRTREEPYATPLVPTRTKGT	+++
pp63-82	PLVPTRTKGTTGSPTEDVFT	–
pp73-92	TGSPTEDVFTVGSTTHSHKG	–
pp83-102	VGSTTHSHKGSQSTTTQNVV	–
pp93-112	SQSTTTQNVVTSQSHDKGDE	–
pp103-122	TSQSHDKGDEEKSKTVTKDG	–
pp113-125	EKSKTVTKDGLGT	–

+++, OD655 ≥ 0.6; –, OD655 < 0.1.

Results

We first synthesized a series of pPDPN peptides, which are summarized in Table 1. Using ELISA, PMab-213 detected 43–62 and 53–72 peptides corresponding to the 43rd–62nd and 53rd–72nd aas of pPDPN, respectively. We then synthesized point mutants of 43–62 peptides (Table 2). Using ELISA, PMab-213 detected S43A, P44A, G45A, V46A, E47A, D48A, Y49A, T50A, V51A, T52A, T55A, E57A, E58A, P59A, A61G, and T62A. Conversely, it did not detect P53A, R54A, R56A, and Y60A, indicating that Pro53, Arg54, Arg56, and Tyr60 are critical epitopes of PMab-213.

We then performed a blocking assay using flow cytometry. PMab-213 reacted with the PK-15 cell line (Fig. 1A). This reaction was completely neutralized by S43A. Conversely, P53A, R54A, R56A, and Y60A did not block the reaction of PMab-213 with PK-15. Similarly, PMab-213 reacted with the CHO/pPDPN cell line, and this reaction was partially neutralized by S43A (Fig. 1B). Conversely, P53A, R54A, R56A, and Y60A did not block the reaction of PMab-213 with CHO/pPDPN, confirming that Pro53, Arg54, Arg56, and Tyr60 of pPDPN are critical for PMab-213 detection.

Table 2. Determination of PMab-213 Epitope by Enzyme-Linked Immunosorbent Assay

Mutation	*Sequence*	*PMab-213*
S43A	**A**PGVEDYTVTPRTREEPYAT	+++
P44A	S**A**GVEDYTVTPRTREEPYAT	+++
G45A	SP**A**VEDYTVTPRTREEPYAT	+++
V46A	SPG**A**EDYTVTPRTREEPYAT	+++
E47A	SPGV**A**DYTVTPRTREEPYAT	+++
D48A	SPGVE**A**YTVTPRTREEPYAT	+++
Y49A	SPGVED**A**TVTPRTREEPYAT	+++
T50A	SPGVEDY**A**VTPRTREEPYAT	+++
V51A	SPGVEDYT**A**TPRTREEPYAT	+++
T52A	SPGVEDYTV**A**PRTREEPYAT	+++
P53A	SPGVEDYTVT**A**RTREEPYAT	–
R54A	SPGVEDYTVTP**A**TREEPYAT	–
T55A	SPGVEDYTVTPR**A**REEPYAT	+++
R56A	SPGVEDYTVTPRT**A**EEPYAT	–
E57A	SPGVEDYTVTPRTR**A**EPYAT	+++
E58A	SPGVEDYTVTPRTRE**A**PYAT	+++
P59A	SPGVEDYTVTPRTREE**A**YAT	+++
Y60A	SPGVEDYTVTPRTREEP**A**AT	–
A61G	SPGVEDYTVTPRTREEPY**G**T	+++
T62A	SPGVEDYTVTPRTREEPYA**A**	+++

Bold indicates the replaced amino acids.
+++, OD655 ≥ 0.6; –, OD655 < 0.1.

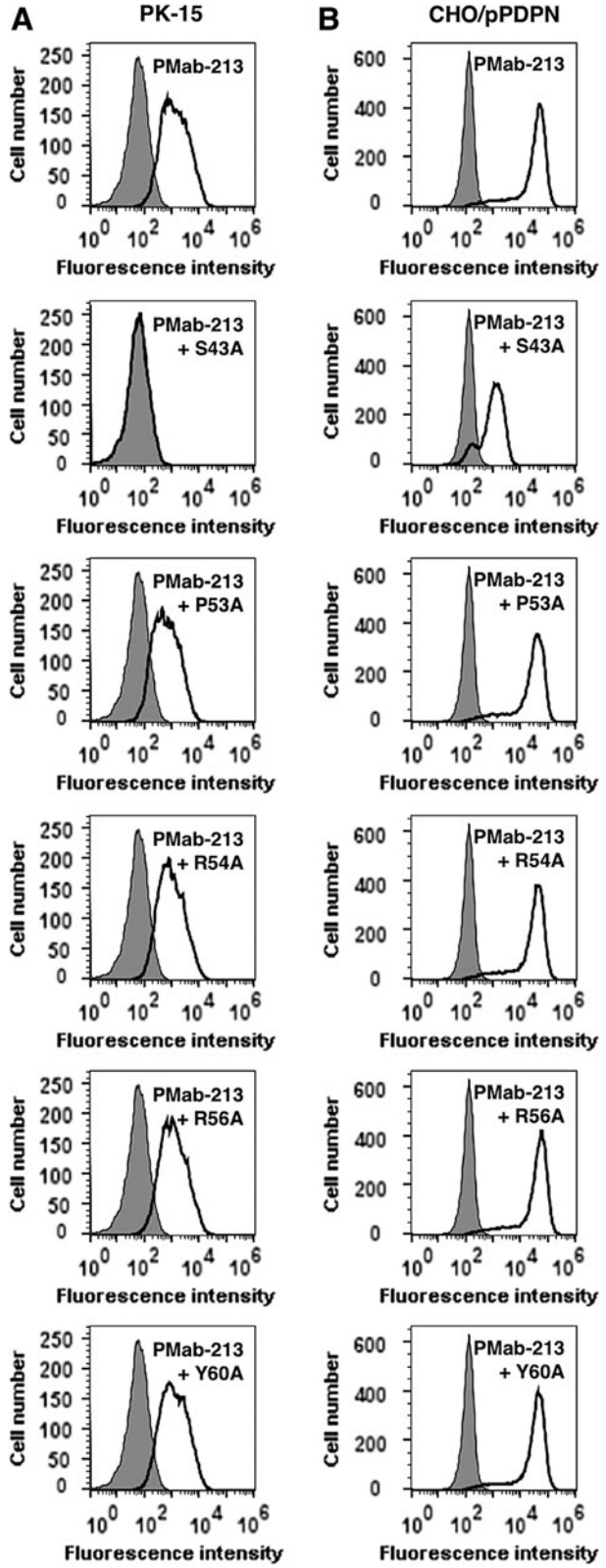

FIG. 1. Flow cytometry using PMab-213 and point mutants of pPDPN. PMab-213 (1 μg/mL), PMab-213 (1 μg/mL) plus peptides (10 μg/mL), or control (without primary mAbs; gray) was reacted with **(A)** PK-15 or **(B)** CHO/pPDPN cells for 30 min at 4°C, followed by the addition of secondary antibodies. CHO, Chinese hamster ovary; mAbs, monoclonal antibodies; pPDPN, pig podoplanin.

We further performed a blocking assay using immunohistochemistry. PMab-213 reacted with the podocytes of the kidneys (Fig. 2). These reactions were completely neutralized by S43A. Conversely, Y60A (Fig. 2) and P53A, R54A, and R56A (data not shown) did not block these reactions of PMab-213. These data confirmed that Pro53, Arg54, Arg56, and Tyr60 of pPDPN are critical for PMab-213 detection.

Discussion

We have previously produced several mAbs against CD44, PD-L1, and CD133 using the CBIS method.[26,28–31] However, the binding epitopes of these mAbs (C_{44}Mab-5 against CD44, L_1Mab-13 against PD-L1, and CMab-43 against CD133)

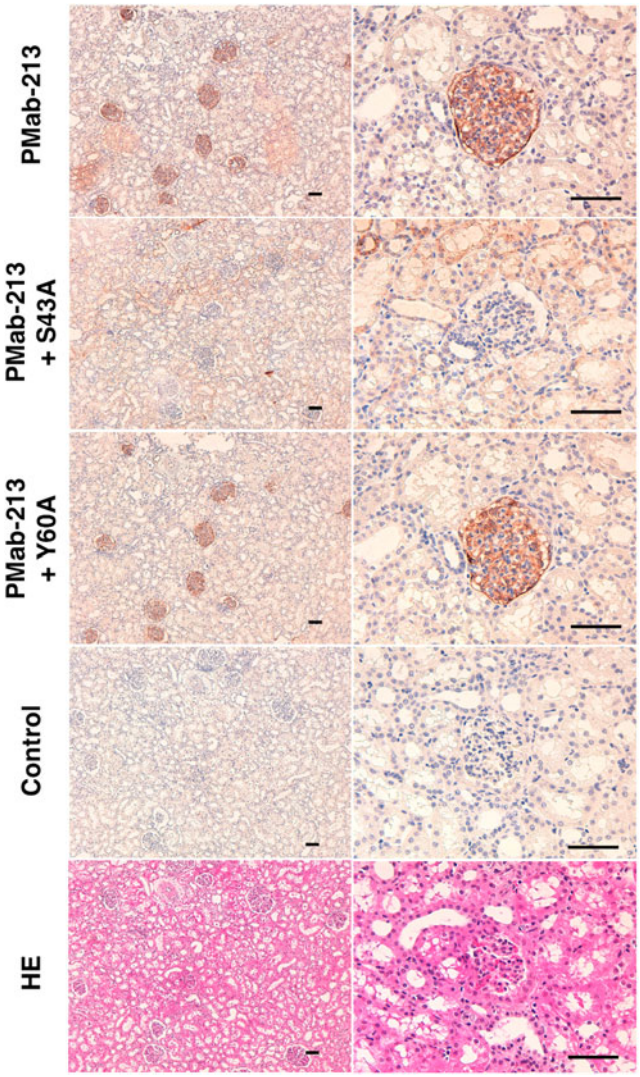

FIG. 2. Immunohistochemical analyses using PMab-213 and point mutants of pPDPN. Histological sections of the kidneys were directly autoclaved in citrate buffer for 20 min. After blocking with SuperBlock T20 (PBS) blocking buffer, the created sections were incubated with PMab-213 (1 μg/mL), PMab-213 (1 μg/mL) + peptides (1 μg/mL), or control (blocking buffer), followed by treatment with the Envision + Kit. Scale bar: 100 μm. HE, hematoxylin and eosin; PBS, phosphate-buffered saline.

remain to be determined because these mAbs did not react with synthesized peptides. Conversely, the binding epitopes of mAbs produced by immunizing with synthesized peptides have been easily determined using deletion mutants and point mutants of synthesized peptides.[32,33] Although PMab-213 was produced using the CBIS method in this study, the epitope of PMab-213 was readily determined by ELISA (Tables 1 and 2).

To date, we have developed mAbs against human,[34] mouse,[34] rat,[35] rabbit,[36] dog,[37] cat,[30] bovine,[38] horse,[27,39,40] and pig[25] PDPNs. PDPN comprises three tandem repeats of the ''EDxxVTPG'' sequences, which were defined to be platelet aggregation-stimulating (PLAG) domains (PLAG1, PLAG2, and PLAG3) in the N-terminus of the PDPN protein.[4] There are several PLAG-like domains (PLDs) of the ''E(D/E)xx(T/S)xx'' sequence in the middle of the PDPN protein. PLDs are reportedly important for PDPN–CLEC-2 interactions.[41] Almost all mAbs against PDPNs reportedly react with PLAG domains or PLDs.[41,42] Flow cytometry (Fig. 1) and immunohistochemical analyses (Fig. 2) further confirmed Pro53, Arg54, Arg56, and Tyr60 of pPDPN (Table 2) as the critical epitope of PMab-213 in this study. As summarized in Figure 3, Pro53 and Arg54 are included in the PLAG3 domain, whereas Arg56 and Tyr60 are included in the first PLD. Therefore, PLAG3 and the adjoining PLD of pPDPN were also clarified to be advantageous epitopes for several applications, such as flow cytometry, Western blotting, and immunohistochemical analyses.

PMab-213 showed no reaction with human, mouse, rat, rabbit, dog, cat, bovine, horse, Tasmanian devil, tiger, alpaca, bear, goat, sheep, or whale PDPNs, thereby indicating that PMab-213 is specific to pPDPN.[25] Indeed, the corresponding sequence of PMab-213 ''PRTREEPY'' of human PDPN is ''PGTSEDRY,'' which indicates that only two of the four aas are the same (share 50% homology). Similarly, ''TGATGGLN'' of mouse PDPN (0/4 aas: 0% homology), ''TDTTGELD'' of rat PDPN (0/4 aas: 0% homology), ''DGATEEPY'' of dog PDPN (1/4 aas: 25% homology), and ''PAASKESL'' of bovine PDPN (1/4 aas: 25% homology) are different from the corresponding sequence of the PMab-213 epitope.[4]

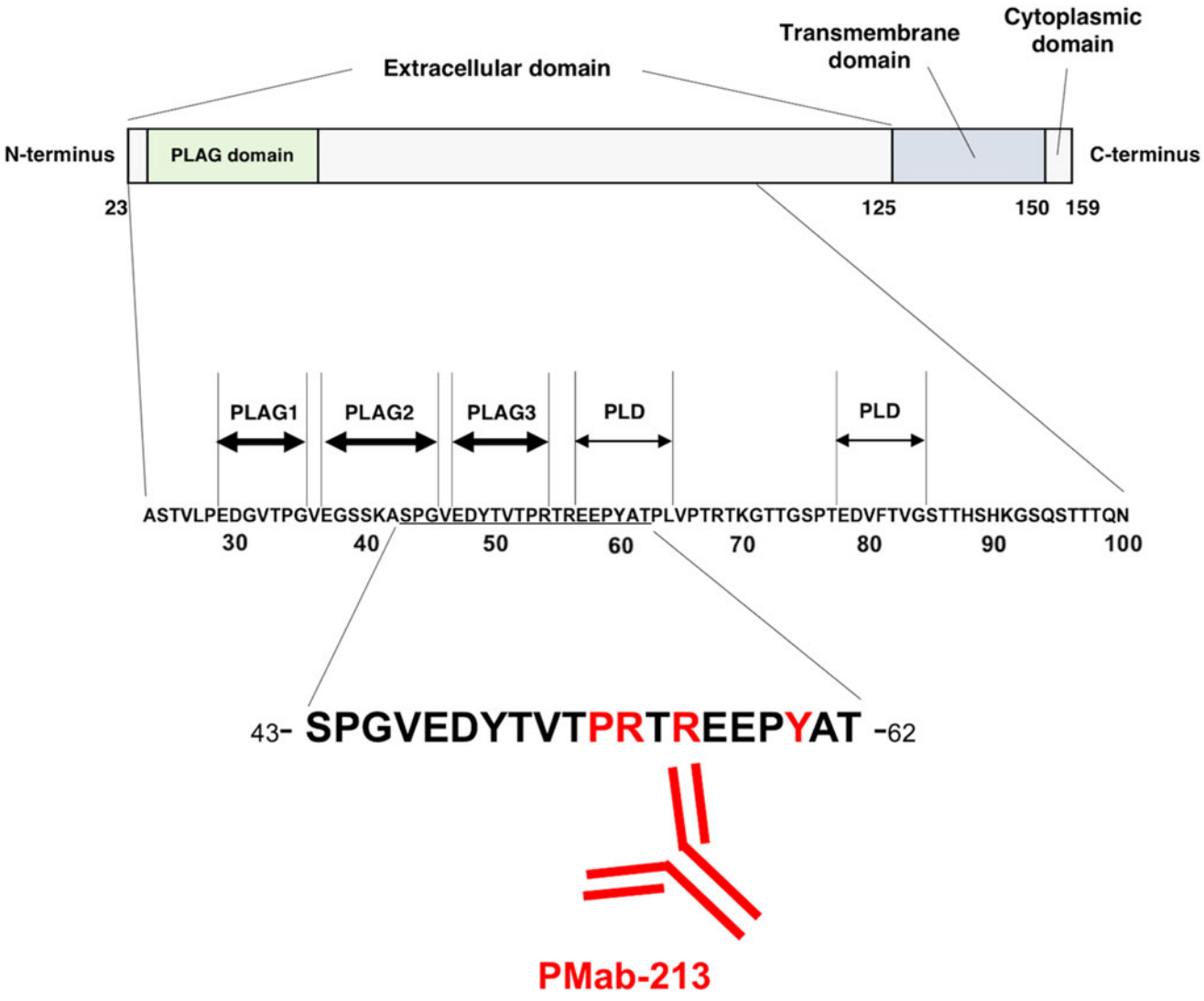

FIG. 3. Schematic illustration of the epitope recognized by PMab-213. PLAG, platelet aggregation-stimulating; PLD, PLAG-like domain.

In summary, the critical epitopes of PMab-213 are Pro53, Arg54, Arg56, and Tyr60 of pPDPN. Among these epitopes, Pro53 and Arg54 are included in the PLAG3 domain. Ser/Thr residues, which are involved in *O*-glycosylation, are not included in PMab-213 epitope, indicating that PMab-213 was not categorized into GpMabs.[42] Conversely, PLAG3 and the adjoining PLD of pPDPN were clarified to be advantageous epitopes for several applications. These findings can be applied to the production of more functional anti-pPDPN mAbs.

Author Disclosure Statement

No competing financial interests exist.

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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. 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.
12. 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.
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. 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.
17. Kimura N, and Kimura I: Podoplanin as a marker for mesothelioma. Pathol Int 2005;55:83–86.
18. 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.
19. 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.
20. 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.
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. 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.
25. 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.
26. 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.
27. 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.
28. 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.
29. Yamada S, Itai S, Kaneko MK, and Kato Y: Detection of high PD-L1 expression in oral cancers by a novel monoclonal antibody L1Mab-4. Biochem Biophys Rep 2018;13:123–128.
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. 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.
32. Furusawa Y, Yamada S, Itai S, Nakamura T, Fukui M, Harada H, Kaneko MK, and Kato Y: Elucidation of critical epitope of anti-rat podoplanin monoclonal antibody PMab-2. Monoclon Antib Immunodiagn Immunother 2018;37:188–193.
33. Yamada S, Itai S, Kaneko MK, Konnai S, and Kato Y: Epitope mapping of anti-mouse podoplanin monoclonal antibody PMab-1. Biochem Biophys Rep 2018;15:52–56.
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. 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.
36. 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.
37. 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.
38. 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.
39. 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.
40. 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 2019;18. DOI:10.1016/j.bbrep.2019.1001.1009
41. 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.
42. 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.

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