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Original Articles

Expression of Cat Podoplanin in Feline Squamous Cell Carcinomas

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Oral squamous cell carcinoma is an aggressive tumor in cats; however, molecular-targeted therapies against this tumor, including antibody therapy, have not been developed. Sensitive and specific monoclonal antibodies (mAbs) against highly expressed membrane proteins are needed to develop antibody therapies. Podoplanin, a type I transmembrane glycoprotein, is expressed in many human malignant tumors, including brain tumor, esophageal cancer, lung cancer, mesothelioma, and oral cancer. Podoplanin binds to C-type lectin-like receptor-2 (CLEC-2) and activates platelet aggregation, which is involved in cancer metastasis. Until now, we have established several mAbs against podoplanin in humans, mice, rats, rabbits, dogs, cattle, and cats. We have reported podoplanin expression in canine melanoma and squamous cell carcinomas using an anti-dog podoplanin mAb PMab-38. In this study, we investigated podoplanin expression in 40 feline squamous cell carcinomas (14 cases of mouth floor, 13 of skin, 9 of ear, and 4 of tongue) by immunohistochemical analysis using an anti-cat podoplanin mAb PMab-52, which we recently developed by cell-based immunization and screening (CBIS) method. Of the total 40 cases, 38 (95%) showed positive staining for PMab-52. In particular, 12 cases (30%) showed a strong membrane-staining pattern of squamous cell carcinoma cells. PMab-52 can be useful for antibody therapy against feline podoplanin-expressing squamous cell carcinomas.

Keywords: cat podoplanin, immunohistochemistry, monoclonal antibody, squamous cell carcinoma

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Introduction

Oral squamous cell carcinoma is an aggressive tumor in cats,[1] for which no molecular targeted therapies have been developed. In particular, sensitive and specific monoclonal antibodies (mAbs) should be established for developing antibody-based therapies. Although many mAbs against human membrane proteins have been developed, almost all mAbs do not cross-react with the feline ones. Therefore, we need to develop sensitive and specific mAbs that can be directed against feline membrane proteins, which are overexpressed in feline cancers.

Podoplanin (PDPN/T1α/Aggrus), a type I transmembrane glycoprotein,[2–6] activates platelet aggregation by binding to the C-type lectin-like receptor-2 (CLEC-2) on the platelets.[7–10] Podoplanin is expressed in normal cells, including renal podocytes, pulmonary type I alveolar cells, and lymphatic endothelial cells.[3] The interaction between podoplanin and CLEC-2 facilitates lymphatic/blood vessel separation.[11] The expression of human podoplanin has been reported in many malignant tumors, including brain tumors,[12–15] oral cancers,[16] esophageal cancers,[17] lung cancers,[18] mesotheliomas,[19,20] testicular tumors,[21] osteosarcomas,[22–24] and chondrosarcomas.[23] Podoplanin expression is associated with cancer metastasis and malignant progression.[8,12,25]

We have established several mAbs against human,[26] mouse,[27] rat,[28] rabbit,[29] dog,[30] bovine,[31] and cat podoplanin.[32] Using these antipodoplanin mAbs, we have investigated podoplanin expression in cancer tissues. PMab-38, an anti-dog podoplanin mAb, reacted with 83% of squamous cell carcinomas (15/18 cases)[33] and 90% of melanomas (9/10 cases).[34] Recently, we have established an anti-cat podoplanin mAb (clone: PMab-52), which is very useful in flow cytometry, Western blot, and immunohistochemical analyses.[32]

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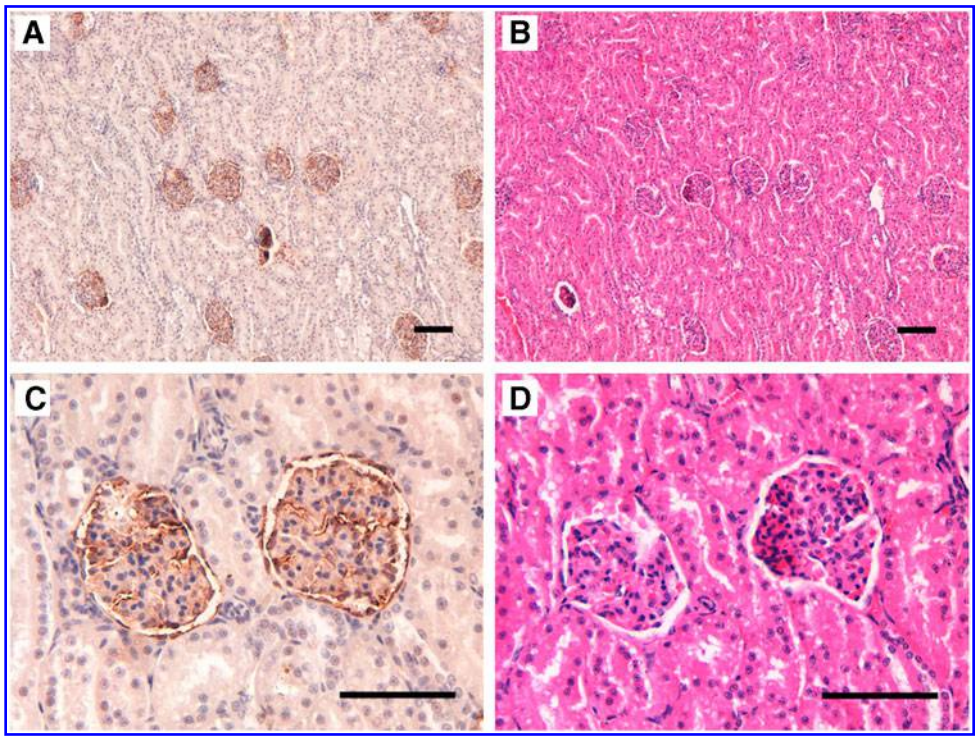

FIG. 1. Immunohistochemical analysis of podoplanin using PMab-52 in feline kidney. **(A, C)** Sections of feline kidneys were autoclaved in citrate buffer (pH 6.0). After blocking, they were incubated with 1 μg/mL of PMab-52, followed by treatment with EnVision+ kit. Color was developed using DAB, and the slides were counterstained with hematoxylin. **(B, D)** H&E staining was performed against serial sections. Scale bar: 100 μm. DAB, 3, 3-diaminobenzidine tetrahydrochloride; H&E, hematoxylin and eosin.

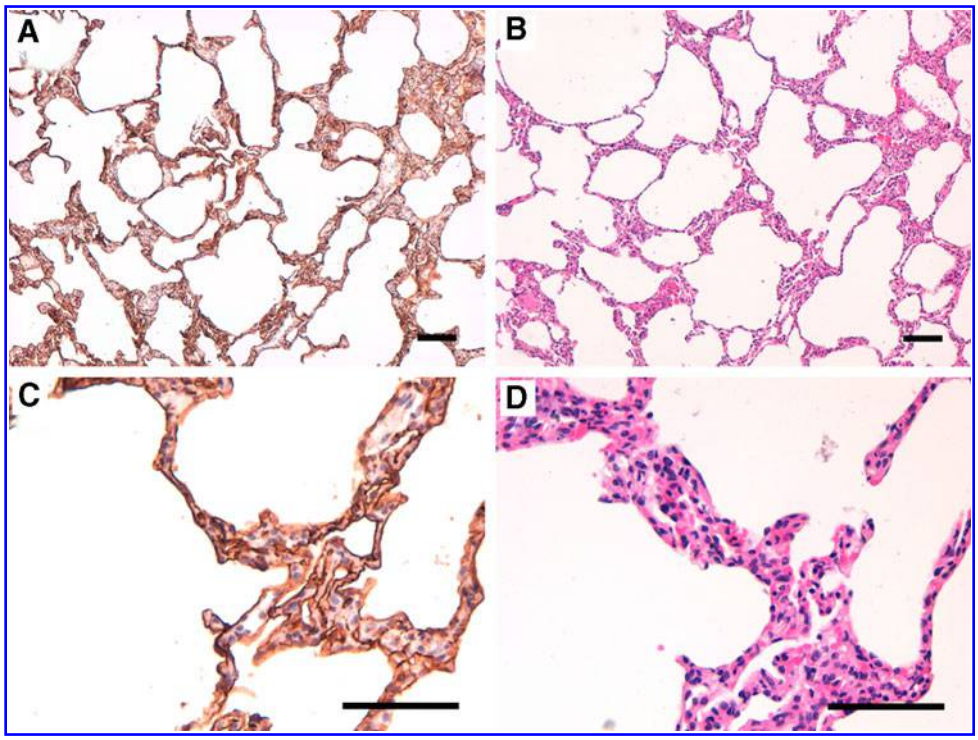

FIG. 2. Immunohistochemical analysis of podoplanin using PMab-52 in feline lungs. **(A, C)** Sections of feline lungs were autoclaved in citrate buffer (pH 6.0). After blocking, they were incubated with 1 μg/mL of PMab-52, followed by treatment with EnVision+ kit. Color was developed using DAB, and the slides were counterstained with hematoxylin. **(B, D)** H&E staining was performed against serial sections. Scale bar: 100 μm.

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In this study, we investigated podoplanin expression in feline squamous cell carcinomas by immunohistochemical analysis using PMab-52.

Materials and Methods

Feline tissues

Normal feline tissues were purchased from Zyagen (San Diego, CA). Feline squamous cell carcinoma tissues were obtained from North Lab (Hokkaido, Japan).

Hybridoma production

In brief, BALB/c mice (CLEA Japan, Tokyo, Japan) were immunized by intraperitoneal (i.p.) injection of cat podoplanin-expressing CHO cells (CHO/cat podoplanin), which were produced by transfecting cat podoplanin into CHO-K1 cells[32] [American Type Culture Collection (ATCC), Manassas, VA] along with Imject Alum (Thermo Fisher Scientific, Inc., Waltham, MA). After several additional immunizations of CHO/cat podoplanin cells, a booster injection of CHO/cat podoplanin cells was intraperitoneally administered 2 days before the spleen cells were harvested. The spleen cells were fused with P3U1 cells (ATCC) using PEG1500 (Roche Diagnostics, Indianapolis, IN). The hybridomas were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Inc., Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Inc.) and hypoxanthine, aminopterin, and thymidine selection medium supplement (Thermo Fisher Scientific, Inc.). The culture supernatants were screened using flow cytometry for binding to CHO/cat podoplanin and CHO-K1.

Immunohistochemical analyses

First, 4-μm-thick histological sections were deparaffinized in xylene and then rehydrated, followed by autoclaving in citrate buffer (pH 6.0; Agilent Technologies, Inc., Santa Clara, CA) for 20 minutes. The sections were incubated with 1 μg/mL of PMab-52 for 1 hour at room temperature, followed by treatment with Envision+ kit for 30 minutes (Agilent Technologies, Inc.). Color was developed using 3, 3-diaminobenzidine tetrahydrochloride (Agilent Technologies, Inc.) for 2 minutes, and the sections were counterstained with hematoxylin (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The intensity of staining was evaluated as 0, 1+, 2+, and 3+.

Results

In our recent studies, we immunized mice with CHO/cat podoplanin cells and performed flow cytometry as the first screening. Using this cell-based immunization and screening (CBIS) method, we could obtain highly sensitive mAbs, which are useful not only in flow cytometry but also in Western blot and immunohistochemical analyses. Indeed, one of the established clones, PMab-52 (IgM, kappa), was sensitive and specific against cat podoplanin in flow cytometry, Western blot, and immunohistochemical analyses. PMab-52 reacted with only cat podoplanin, whereas it did not react with human, mouse, rat, rabbit, dog, and bovine podoplanin in flow cytometry and

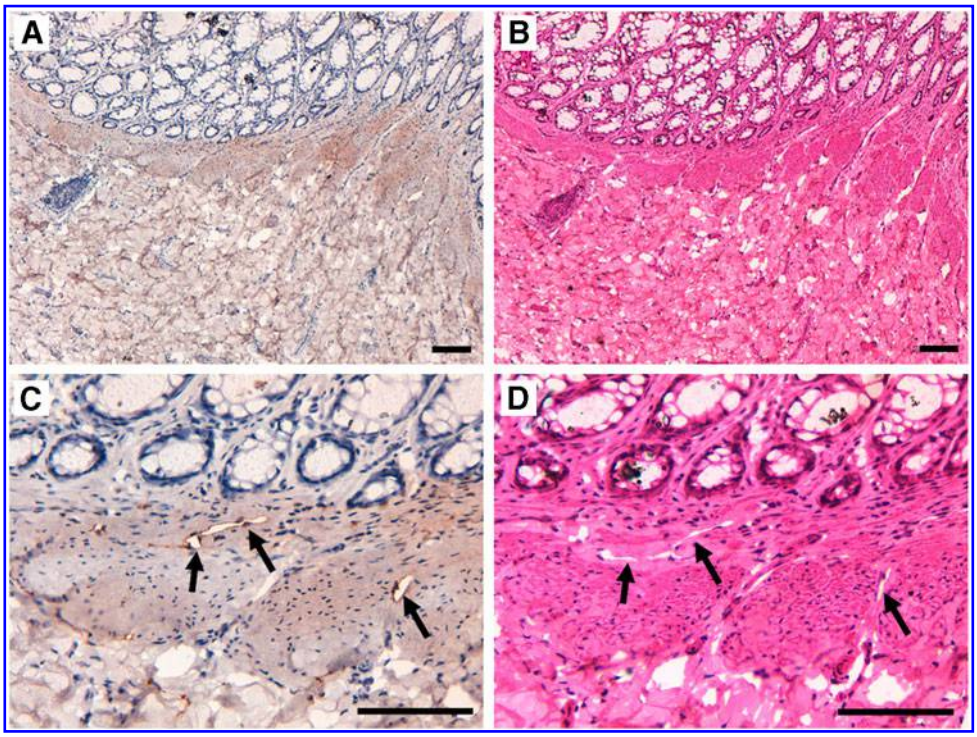

FIG. 3. Immunohistochemical analysis of podoplanin using PMab-52 in feline rectum. **(A, C)** Sections of feline rectum were autoclaved in citrate buffer (pH 6.0). After blocking, they were incubated with 1 μg/mL of PMab-52, followed by treating with EnVision+ kit. Color was developed using DAB, and the slides were counterstained with hematoxylin. **(B, D)** H&E staining was performed against serial sections. Arrows: lymphatic endothelial cells. Scale bar: 100 μm.

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Table 1. Results of PMab-52 Immunostaining in 40 Feline Squamous Cell Carcinomas

No.	*Site*	*Breed*	*Age (year)*	*Sex (reproductive status)*	*PMab-52 (IHC)*
1	Skin	Mix	13	F (spayed)	1+
2	Ear	Mix	10	F (spayed)	1+
3	Ear	Mix	16	F (spayed)	3+
4	Tongue	Scottish Fold	10	F (spayed)	3+
5	Floor of mouth	Mix	16	F (spayed)	1+
6	Floor of mouth	Mix	13	F (spayed)	2+
7	Tongue	Mix	13	F (spayed)	1+
8	Floor of mouth	Mix	15	M (casted)	2+
9	Skin	Mix	11	M (casted)	3+
10	Skin	Mix	15	M (casted)	3+
11	Skin	Mix	14	F (spayed)	3+
12	Floor of mouth	Mix	14	F (spayed)	1+
13	Ear	Mix	16	F (entire)	1+
14	Skin	Mix	11	M (casted)	1+
15	Skin	Mix	7	M (casted)	2+
16	Ear	Mix	16	F (spayed)	2+
17	Floor of mouth	Mix	11	F (spayed)	2+
18	Ear	Mix	17	F (spayed)	1+
19	Ear	Mix	15	F (spayed)	2+
20	Ear	Mix	14	F (spayed)	1+
21	Ear	Mix	Not specified	F (entire)	0
22	Floor of mouth	Mix	13	F (spayed)	2+
23	Floor of mouth	Persian	12	F (spayed)	3+
24	Skin	Mix	14	F (entire)	2+
25	Floor of mouth	Maine Coon	Not specified	Not specified	1+
26	Tongue	Mix	16	M (entire)	2+
27	Floor of mouth	American Shorthair	14	M (casted)	2+
28	Floor of mouth	Mix	15	M (casted)	0
29	Skin	Mix	16	M (casted)	3+
30	Skin	Mix	9	M (casted)	3+
31	Skin	Mix	10	F (spayed)	2+
32	Floor of mouth	Persian	10	M (casted)	3+
33	Skin	Mix	10	F (spayed)	3+
34	Floor of mouth	American Shorthair	13	M (casted)	1+
35	Skin	Mix	Not specified	F (entire)	1+
36	Skin	Mix	12	F (spayed)	3+
37	Floor of mouth	Mix	16	F (spayed)	1+
38	Floor of mouth	Persian	13	M (entire)	2+
39	Ear	Mix	11	F (spayed)	2+
40	Tongue	Not specified	15	F (spayed)	3+

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Western blot analyses.[32] Although we have investigated podoplanin expression in human, rabbit, and canine cancers, its expression in feline cancer remains to be clarified.

First, we investigated podoplanin expression in normal feline tissues, including the kidney, lung, and rectum, by immunohistochemical analysis. PMab-52 was bound to the Bowman's capsule and glomerular podocytes in feline kidneys (Fig. 1A, C). Also, it was bound to the type I alveolar cells of feline lungs (Fig. 2A, C) and lymphatic endothelial cells (Fig. 3A, C), indicating that PMab-52 can detect cat podoplanin in normal tissues because a similar expression pattern has been reported in other species, including human and mouse.[35]

Next, we investigated cat podoplanin expression in various squamous cell carcinomas because its expression has been

Table 2. Summary of PMab-52 Immunostaining in 40 Feline Squamous Cell Carcinomas

		PMab-52 immunostaining					
Site	*No. of cases*	*3+*	*2+*	*1+*	*0*	*No. of positive cases*	*Positive rate (%)*
Floor of mouth	14	2	6	5	1	13/14	92.9
Skin	13	7	3	3	0	13/13	100
Ear	9	1	3	4	1	8/9	88.9
Tongue	4	2	1	1	0	4/4	100
Total	40	12	13	13	2	38/40	95.0

reported in many previous studies with other species.[18,33] All specimens of squamous cell carcinomas used in this study are listed in Table 1. Among the 40 cases analyzed, 14 were from mouth floor, 13 from skin, 9 from ear, and 4 from tongue (Table 2). PMab-52 was observed in squamous cell carcinoma cells in a membrane-staining pattern (Fig. 4). All staining patterns are depicted in Supplementary Figure S1 and S2 (Supplementary Data are available online at www.liebertpub.com/mab). In total, 95% (38/40) of squamous cell carcinomas were detected using PMab-52 (Table 2).

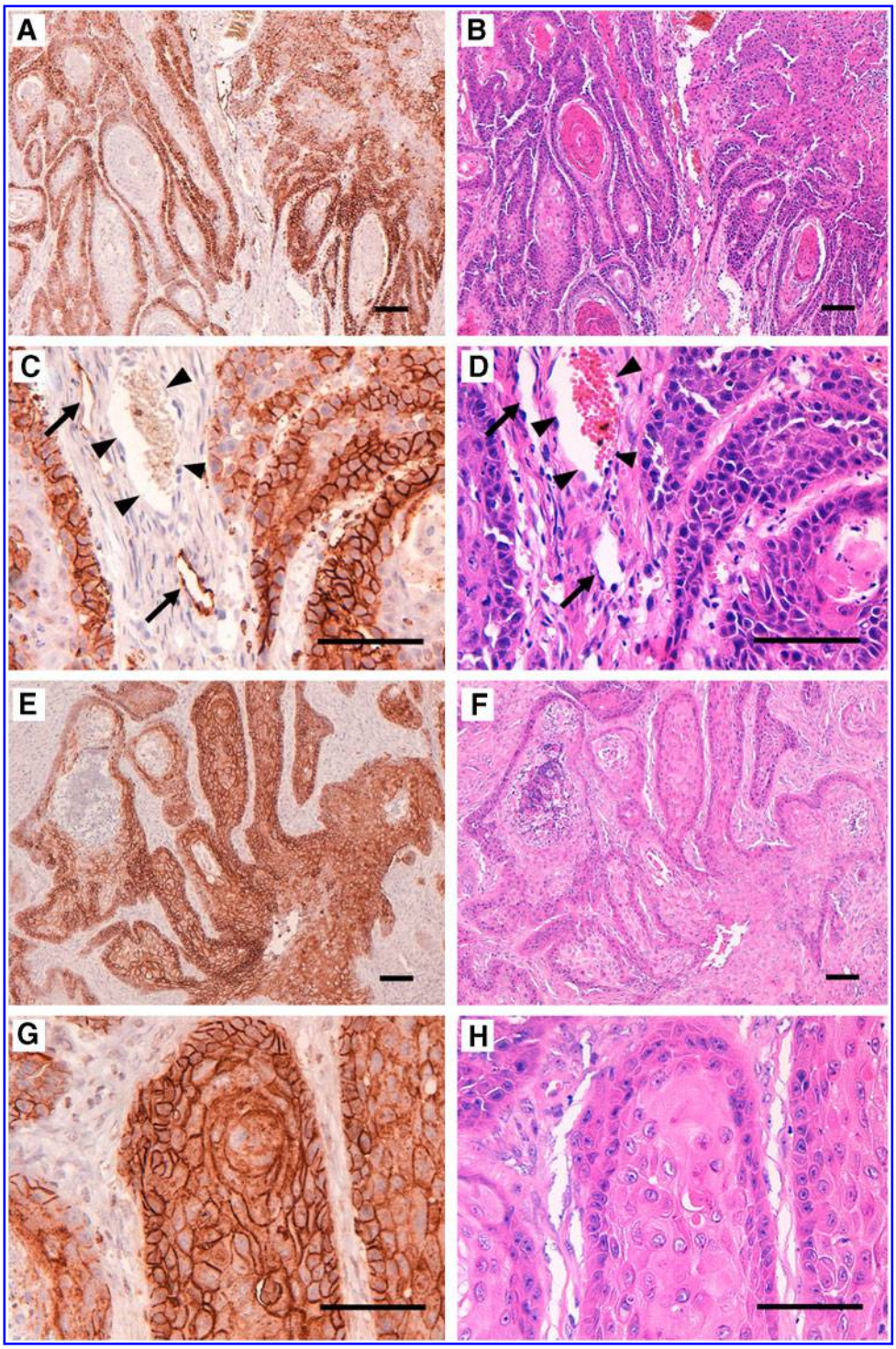

FIG. 4. Immunohistochemical analysis of cat podoplanin using PMab-52 in squamous cell carcinomas. **(A, C, E, G)** Sections of feline squamous cell carcinomas were autoclaved in citrate buffer (pH 6.0). After blocking, they were incubated with 1 μg/ml of PMab-52, followed by treatment with EnVision+ kit. Color was developed using DAB, and the slides were counterstained with hematoxylin. **(B, D, F, H)** H&E staining was performed against serial sections. **(A–D)** case 9; **(E–H)** case 11. Arrows: lymphatic endothelial cells; arrowheads: vascular endothelial cells. Scale bar: 100 μm.

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PMab-52 also detected lymphatic endothelial cells around the squamous cell carcinoma cells, but it could not detect vascular endothelial cells (Fig. 4C). PMab-52 very weakly detected normal lymphatic endothelial cells (Fig. 3), indicating that the expression level of podoplanin is different between normal and cancerous tissues.

Discussion

In this study, we first investigated podoplanin expression in feline normal tissues. PMab-52 strongly bound to feline kidneys (Fig. 1) and lungs (Fig. 2). These results are consistent with our previous study regarding podoplanin expression in rabbit normal tissues.[29] In contrast, anti-dog podoplanin mAb PMab-38 reacted with canine kidney, but not with canine lungs.[30] PMab-52 was weakly bound to normal lymphatic endothelial cells (Fig. 3), although podoplanin is known to be a specific marker for lymphatic endothelial cells. Likewise, PMab-38 did not detect normal lymphatic endothelial cells of canine normal tissues.[30] NZ-1, an anti-human podoplanin mAb, detects lymphatic endothelial cells but not those of the lung and kidney.[26] Moreover, cancer-specific mAbs LpMab-2[15] and LpMab-23[36] do not react with human podoplanin in all normal tissues. Taken together, two main reasons can explain the different reaction patterns of anti-podoplanin mAbs: (1) the expression level of podoplanin is different among tissues and (2) the epitopes recognized by antipodoplanin mAbs vary due to many reasons, including tissue-specific posttranslational modification or cancer-specific glycosylation.

We investigated cat podoplanin expression in 40 squamous cell carcinoma tissues. PMab-52 strongly detected lymphatic endothelial cells around squamous cell carcinoma cells (Fig. 4C), whereas it weakly detected normal lymphatic endothelial cells (Fig. 3C), indicating that the expression level of podoplanin differs between normal and cancer tissues. We have previously reported that podoplanin expression can be induced by transforming growth factor-β,[37] which might explain the different expression levels of podoplanin in cancer tissues. Cancer-associated fibroblasts were also detected in some cases (case 14; Supplementary Fig. S1), which have been observed in many previous studies using anti-podoplanin mAbs in human cancer tissues[38–40] and using PMab-38 in canine squamous cell carcinomas.[33] Targeted therapy against cancer-associated fibroblasts is also important because cancer-associated fibroblasts have been associated with malignancy.[38–40]

In conclusion, PMab-52 can be useful for clarifying the pathophysiological function of podoplanin in feline squamous cell carcinomas. Furthermore, it is a potential candidate for developing antibody-based therapies against feline squamous cell carcinomas.

Author Disclosure Statement

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References

1. Gardner DG: Spontaneous squamous cell carcinomas of the oral region in domestic animals: A review and consideration of their relevance to human research. Oral Dis 1996; 2:148–154.
2. 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.
3. 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.
4. Kato Y, Fujita N, Kunita A, Sato S, Kaneko M, Osawa M, and Tsuruo T: Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. J Biol Chem 2003;278:51599–51605.
5. 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.
6. 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.
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. 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.
9. 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.
10. 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.
11. 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:

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Platelets regulate lymphatic vascular development through CLEC-2-SLP-76 signaling. Blood 2010;116:661–670.
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. 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.
17. 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.
18. 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.
19. Kimura N, and Kimura I: Podoplanin as a marker for mesothelioma. Pathol Int 2005;55:83–86.
20. 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.
21. 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.
22. 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.
23. 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.
24. 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.
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. Kato Y, Kaneko MK, Kuno A, Uchiyama N, Amano K, Chiba Y, Hasegawa Y, Hirabayashi J, Narimatsu H, Mishima K, and Osawa M: Inhibition of tumor cell-induced platelet aggregation using a novel anti-podoplanin antibody reacting with its platelet-aggregation-stimulating domain. Biochem Biophys Res Commun 2006;349:1301–1307.
27. Yamada S, Kaneko MK, Nakamura T, Ichii O, Konnai S, and Kato Y: Development of mPMab-1, a mouse-rat chimeric antibody Against Mouse Podoplanin. Monoclon Antib Immunodiagn Immunother 2017;36:77–79.
28. 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.
29. 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.
30. 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.
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, 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.
33. Kaneko MK, Honma R, Ogasawara S, Fujii Y, Nakamura T, Saidoh N, Takagi M, Kagawa Y, Konnai S, and Kato Y: PMab-38 recognizes canine podoplanin of squamous cell carcinomas. Monoclon Antib Immunodiagn Immunother 2016;35:263–266.
34. Ogasawara S, Honma R, Kaneko MK, Fujii Y, Kagawa Y, Konnai S, and Kato Y: Podoplanin expression in canine melanoma. Monoclon Antib Immunodiagn Immunother 2016;35:304–306.
35. 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.
36. Yamada S, Kaneko MK, and Kato Y: LpMab-23: A cancer-specific monoclonal antibody against human podoplanin. Monoclon Antib Immunodiagn Immunother 2017;36: 72–76.
37. Suzuki H, Kato Y, Kaneko MK, Okita Y, Narimatsu H, and Kato M: Induction of podoplanin by transforming growth factor-beta in human fibrosarcoma. FEBS Lett 2008;582: 341–345.
38. Takahashi A, Ishii G, Kinoshita T, Yoshida T, Umemura S, Hishida T, Yoh K, Niho S, Goto K, Ohmatsu H, Ohe Y, Nagai K, and Ochiai A: Identification of prognostic im-

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munophenotypic features in cancer stromal cells of high-grade neuroendocrine carcinomas of the lung. J Cancer Res Clin Oncol 2013;139:1869–1878.
39. Shindo K, Aishima S, Ohuchida K, Fujino M, Mizuuchi Y, Hattori M, Ohtsuka T, Tokunaga S, Mizumoto K, Tanaka M, and Oda Y: Podoplanin expression in the cyst wall correlates with the progression of intraductal papillary mucinous neoplasm. Virchows Arch 2014;465: 265–273.
40. Ono S, Ishii G, Nagai K, Takuwa T, Yoshida J, Nishimura M, Hishida T, Aokage K, Fujii S, Ikeda N, and Ochiai A: Podoplanin-positive cancer-associated fibroblasts could have prognostic value independent of cancer cell phenotype in stage I lung squamous cell carcinoma: Usefulness of combining analysis of both cancer cell phenotype and cancer-associated fibroblast phenotype. Chest 2013;143: 963–970.

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