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Immunohistochemical Analysis Using Monoclonal Antibody PMab-269 Against Steller Sea Lion Podoplanin

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Monoclonal antibodies (mAbs) that specifically target podoplanin (PDPN), a marker for type I alveolar cells, are required for immunohistochemical analyses. Anti-PDPN mAbs are available for many species, including human, mouse, rat, rabbit, dog, cat, bovine, pig, Tasmanian devil, alpaca, tiger, whale, goat, horse, bear, sheep, and California sea lion PDPNs. However, no anti-Steller sea lion PDPN (stePDPN) antibody has been developed. Immunohistochemical analysis showed that an anti-California sea lion PDPN mAb (PMab-269) reacted with type I alveolar cells from the Steller sea lion lung, renal glomeruli and Bowman's capsules from kidney, and lymphatic endothelial cells from the colon, indicating that PMab-269 is useful for detecting stePDPN.

Keywords: Steller sea lion, podoplanin, PDPN, PMab-269

Introduction

Steller sea lions (*Eumetopias jubatus*) inhabit the coastal waters in the northern Pacific Rim, stretching from central California to northern Japan.[1] The western stock of the Steller sea lion in the northern Pacific Ocean has reduced by >80% in four decades. This resulted in its listing as an endangered species in 1997. Information on the distributions and cause of the reduction is required for management.[2] The closest relative to the Steller sea lion with a sequenced genome is the California sea lion (*Zalophus californianus*). Both species have a diploid karyotype with 18 chromosomes. Alignment of the Steller sea lion assembly to the California sea lion assembly shows a 99.5% similarity between the two genomes.[3]

Podoplanin (PDPN) is a type I transmembrane mucin-like sialoglycoprotein having a heavily glycosylated N-terminal extracellular domain, a single transmembrane domain, and a short intracellular domain. PDPN is identical to T1α, which encodes an antigen expressed at the apical membrane of lung type I alveolar epithelium.[4,5] The name ''podoplanin'' is derived from its expression in kidney podocytes.[6] In a rat model of human minimal change nephrotic syndrome, the reduced expression of PDPN was observed at the proteinuric stage. The amount of urinary PDPN markedly increased on day 1 of PAN nephropathy.[7]

PDPN expression in lymphatic endothelium was also reported and named as ''E11 antigen.''[8] *PDPN* is one of the most highly expressed lymphatic-specific genes but is not expressed in blood vascular endothelial cells.[9,10] Therefore, PDPN is one of the important markers of lymphatic endothelial cells.

PDPN binds to its receptor, the C-type lectin-like receptor-2, and is involved in platelet aggregation.[11] The platelet aggregation-stimulating (PLAG) domain[12–14] and the PLAG-like domain[15] in PDPN ectodomain play a vital role in platelet aggregation.

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Anti-PDPN monoclonal antibodies (mAbs) have been generated in various species, including human,[16] mouse,[17] rat,[18] rabbit,[19] dog,[20] cat,[21] cow,[22] pig,[23] Tasmanian devil,[24] alpaca,[25] tiger,[26] whale,[27] goat,[28] horse,[29] bear,[30] sheep,[31,32] and California sea lion.[33] All mAbs are available for Western blotting, flow cytometry, and immunohistochemistry.

In this study, immunohistochemistry was performed using Steller sea lion tissues and the reactivity of PMab-269 (developed as an anti-California sea lion PDPN mAb) to Steller sea lion PDPN (stePDPN) was investigated.

Materials and Methods

Immunohistochemical analysis

Normal Steller sea lion tissues obtained from routine necropsies performed at the Laboratory of Veterinary Pathology, the University of Tokyo, fixed in 10% neutral-buffered formalin, were processed to make formalin-fixed paraffin-embedded (FFPE) tissue sections. Histological sections (4 μm thickness) were autoclaved in EnVision FLEX Target Retrieval Solution High pH (Agilent Technologies, Inc., Santa Clara, CA, USA) for 20 minutes. After blocking with SuperBlock T20 (phosphate-buffered saline) Blocking Buffer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), sections were incubated using PMab-269 (5 μg/mL) for 1 hour at room temperature and treated with EnVision+ Kit (Agilent Technologies, Inc.) for 30 minutes. The color was developed using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Agilent Technologies, Inc.) for 2 minutes and counterstained using hematoxylin and eosin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Results

Immunohistochemical analyses

We investigated whether PMab-269 can be used for immunohistochemical analyses using FFPE Steller sea lion tissue sections. The normal lung, kidney, and colon tissue from Steller sea lions were examined, all of which express PDPN in different species, including humans,[16] mouse,[17] rat,[18] rabbit,[19] dog,[20] cat,[21] cow,[22] pig,[23] Tasmanian

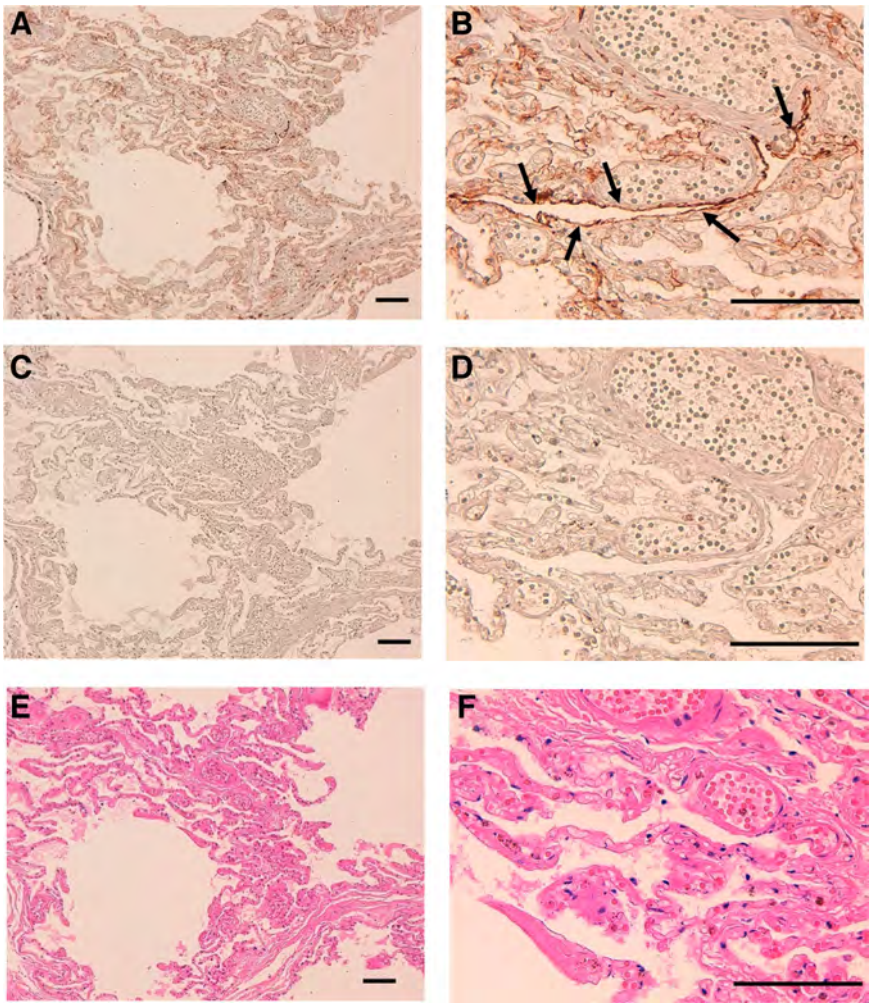

FIG. 1. Immunohistochemical analysis of Steller sea lion lung tissue. Histological sections of Steller sea lion lung were directly autoclaved in EnVision FLEX Target Retrieval Solution High pH and incubated with 5 µg/mL PMab-269 **(A, B)** or blocking buffer **(C, D)**, followed by the use of EnVision+ Kit. **(E, F)** Hematoxylin and eosin staining. *Arrows* indicate the lymphatic endothelial cells. Scale bar = 100 µm.

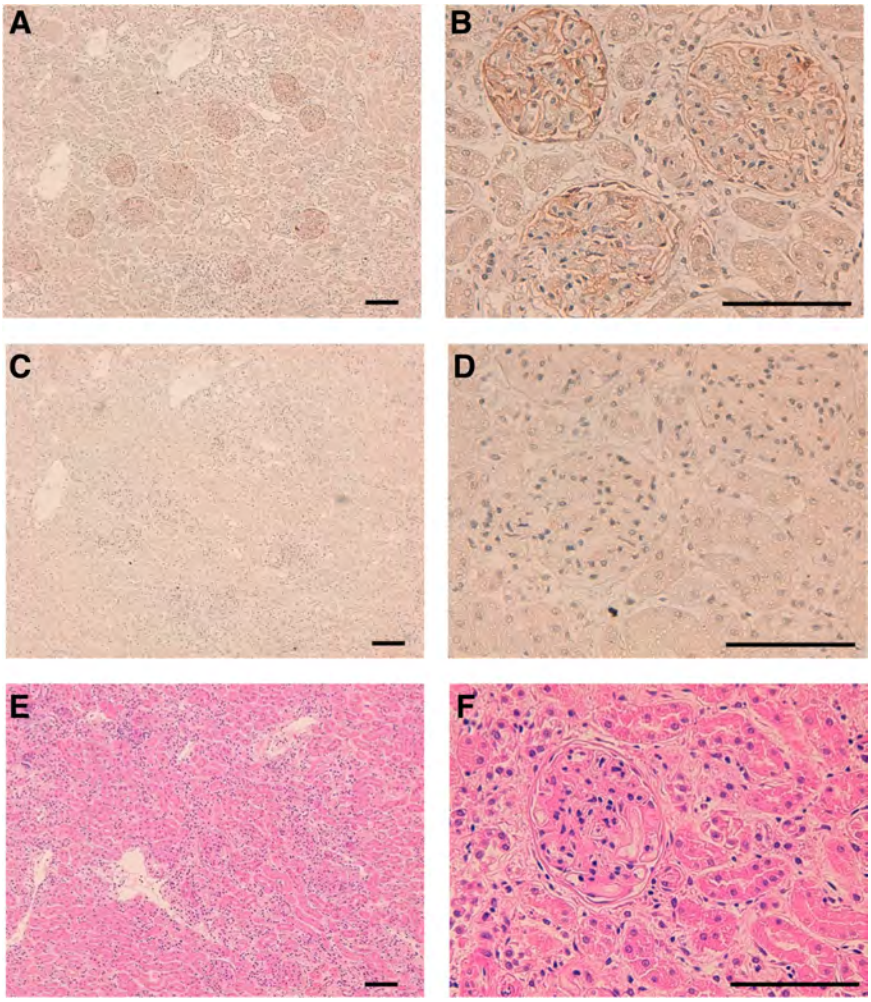

FIG. 2. Immunohistochemical analysis of Steller sea lion kidney tissue. Histological sections of Steller sea lion kidney were directly autoclaved in EnVision FLEX Target Retrieval Solution High pH and incubated with 5 μg/mL PMab-269 **(A, B)** or blocking buffer **(C, D)**, followed by the use of EnVision+ Kit. **(E, F)** Hematoxylin and eosin staining. Scale bar = 100 μm.

devil,[24] alpaca,[25] tiger,[26] whale,[27] goat,[28] horse,[29] bear,[30] sheep,[31,32] and California sea lion.[33] As indicated in Figure 1, PMab-269 strongly and specifically identified type I alveolar cells within the lung. PMab-269 also identified renal glomeruli and Bowman's capsules (Fig. 2). In addition, lymphatic endothelial cells in the lung and submucosa of the colon were detected by PMab-269, respectively (Figs. 1 and 3, arrows). These results show that PMab-269 is useful in detecting stePDPN-positive cells in FFPE tissue.

Discussion

The usefulness of PMab-269 was demonstrated for the pathophysiological analysis of lung, kidney, and lymphatic tissues of the Steller sea lion. PMab-269 was originally developed as an anti-California sea lion PDPN mAb, which has strong cross-reactivity to cat and tiger, but not to the dog PDPN.[33] The binding epitope of PMab-269 as Pro68, Asp76, Phe77, His78, Leu79, Glu80, and Asp81 of California sea lion PDPN was also determined.[34] The sequence is completely identical to that of stePDPN. Alternatively, cat and tiger PDPN match the six of seven amino acids, and dog PDPN matches the five of seven. The Pinnipedia (including sea lions) is assigned to California, which is a suborder of dog-like carnivorans. California stands in contrast to the other suborder of Carnivora, the Feliformia (cat-like carnivorans). At least in the binding epitope of PMab-269, sea lions are similar to the Feliformia.

The number of Steller sea lions of the western stock has reduced. Among several suspected causes for the decline are adverse effects of chemical pollutants, including polychlorinated biphenyls (PCBs). PCBs and their effects on animals have been widely studied.[35–37] PCBs show a broad range of toxic effects, including reproduction failure[38] and immune disorders.[39] Wang et al. investigated 145 individual PCBs in the tissues of sea lions. They determined the mean toxic equivalents in the kidney, liver, and blubber samples, proposing the possibility of adverse effects on animals.[40] PMab-269 might contribute the pathological studies to investigate the tissue damage, including podocyte injury in the kidney.

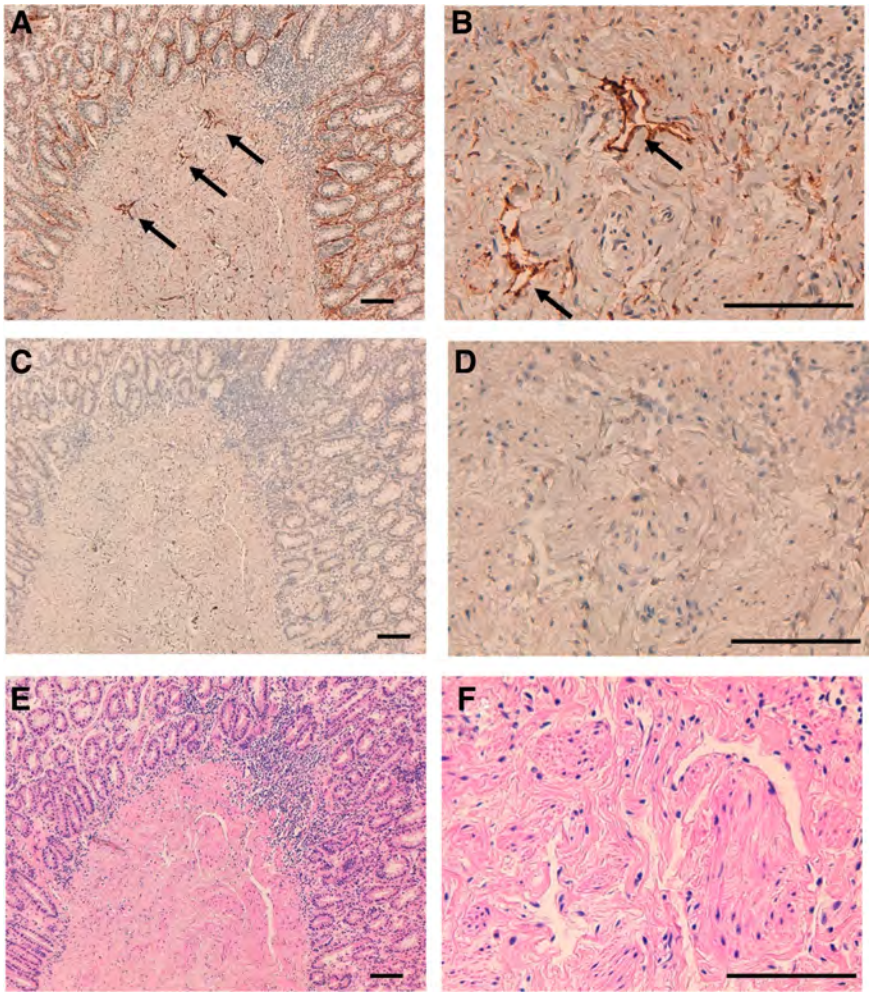

FIG. 3. Immunohistochemical analyses of Steller sea lion colon tissue. Histological sections of Steller sea lion colon were directly autoclaved in EnVision FLEX Target Retrieval Solution High pH and incubated with 5 μg/mL PMab-269 **(A, B)** or blocking buffer **(C, D)**, followed by the use of EnVision + Kit. **(E, F)** Hematoxylin and eosin staining. *Arrows* show the PDPN expressed lymphatic endothelial cells. Scale bar = 100 μm. PDPN, podoplanin.

Author Disclosure Statement

No competing financial interests exist.

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References

1. Ryazanov SD, and Permyakov PA: Steller Sea Lion (*Eumetopias jubatus*) at Tuleny Island, Russia in Autumn 2018: Abundance, composition, and entanglement. Zoolog Sci 2021;38:311–316.
2. Jemison LA, Pendleton GW, Hastings KK, Maniscalco JM, and Fritz LW: Spatial distribution, movements, and geographic range of Steller sea lions (*Eumetopias jubatus*) in Alaska. PLoS One 2018;13:e0208093.
3. Kwan HH, Culibrk L, Taylor GA, Leelakumari S, Tan R, Jackman SD, Tse K, MacLeod T, Cheng D, Chuah E, Kirk H, Pandoh P, Carlsen R, Zhao Y, Mungall AJ, Moore R, Birol I, Marra MA, Rosen DAS, Haulena M, and Jones SJM: The Genome of the Steller Sea Lion (*Eumetopias jubatus*). Genes (Basel) 2019;10:486.
4. Dobbs LG, Williams MC, and Gonzalez R: Monoclonal antibodies specific to apical surfaces of rat alveolar type I cells bind to surfaces of cultured, but not freshly isolated, type II cells. Biochim Biophys Acta 1988;970:146–156.
5. Rishi AK, Joyce-Brady M, Fisher J, Dobbs LG, Floros J, VanderSpek J, Brody JS, and Williams MC: Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. Dev Biol 1995;167:294–306.
6. 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.
7. Suzuki K, Fukusumi Y, Yamazaki M, Kaneko H, Tsuruga K, Tanaka H, Ito E, Matsui K, and Kawachi H: Alteration in the podoplanin-ezrin-cytoskeleton linkage is an impor-

tant initiation event of the podocyte injury in puromycin aminonucleoside nephropathy, a mimic of minimal change nephrotic syndrome. Cell Tissue Res 2015;362:201–213.
8. Wetterwald A, Hoffstetter W, Cecchini MG, Lanske B, Wagner C, Fleisch H, and Atkinson M: Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. Bone 1996;18:125–132.
9. Hirakawa S, Hong YK, Harvey N, Schacht V, Matsuda K, Libermann T, and Detmar M: Identification of vascular lineage-specific genes by transcriptional profiling of isolated blood vascular and lymphatic endothelial cells. Am J Pathol 2003;162:575–586.
10. Petrova TV, Mäkinen T, Mäkelä TP, Saarela J, Virtanen I, Ferrell RE, Finegold DN, Kerjaschki D, Ylä-Herttuala S, and Alitalo K: Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. EMBO J 2002;21:4593–4599.
11. 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.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.
21. 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.
22. 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.
23. 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.
24. Furusawa Y, Yamada S, Itai S, Nakamura T, Takei J, Sano M, Harada H, Fukui M, Kaneko MK, and Kato Y: Establishment of a monoclonal antibody PMab-233 for immunohistochemical analysis against Tasmanian devil podoplanin. Biochem Biophys Rep 2019;18:100631.
25. Kato Y, Furusawa Y, Yamada S, Itai S, Takei J, Sano M, and Kaneko MK: Establishment of a monoclonal antibody PMab-225 against alpaca podoplanin for immunohistochemical analyses. Biochem Biophys Rep 2019;18:100633.
26. Furusawa Y, Kaneko MK, Nakamura T, Itai S, Fukui M, Harada H, Yamada S, and Kato Y: Establishment of a monoclonal antibody PMab-231 for tiger podoplanin. Monoclon Antib Immunodiagn Immunother 2019;38:89–95.
27. Kato Y, Furusawa Y, Itai S, Takei J, Nakamura T, Sano M, Harada H, Yamada S, and Kaneko MK: Establishment of an anticetacean podoplanin monoclonal antibody PMab-237 for immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2019;38:108–113.
28. Furusawa Y, Yamada S, Nakamura T, Sano M, Sayama Y, Itai S, Takei J, Harada H, Fukui M, Kaneko MK, and Kato Y: PMab-235: A monoclonal antibody for immunohistochemical analysis against goat podoplanin. Heliyon 2019;5:e02063.
29. 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.
30. Takei J, Furusawa Y, Yamada S, Nakamura T, Sayama Y, Sano M, Konnai S, Kobayashi A, Harada H, Kaneko MK, and Kato Y: PMab-247 detects bear podoplanin in immunohistochemical analysis. Monoclon Antib Immunodiagn Immunother 2019;38:171–174.
31. Kato Y, Furusawa Y, Sano M, Takei J, Nakamura T, Yanaka M, Okamoto S, Handa S, Komatsu Y, Asano T, Sayama Y, and Kaneko MK: Development of an anti-sheep podoplanin monoclonal antibody PMab-256 for immunohistochemical analysis of lymphatic endothelial cells. Monoclon Antib Immunodiagn Immunother 2020;39:82–90.
32. Kaneko MK, Sano M, Takei J, Asano T, Sayama Y, Hosono H, Kobayashi A, Konnai S, and Kato Y: Development and characterization of anti-sheep podoplanin monoclonal antibodies PMab-253 and PMab-260. Monoclon Antib Immunodiagn Immunother 2020;39:144–155.
33. Tanaka T, Asano T, Sano M, Takei J, Hosono H, Nanamiya R, Nakamura T, Yanaka M, Harada H, Fukui M, Suzuki H, Uchida K, Nakagawa T, Kato Y, and Kaneko MK: Development of monoclonal antibody PMab-269 against California Sea Lion podoplanin. Monoclon Antib Immunodiagn Immunother 2021;40:124–133.

34. Tanaka T, Asano T, Sano M, Takei J, Hosono H, Nanamiya R, Tateyama N, Kaneko MK, and Kato Y: Epitope mapping of the anti-California Sea Lion podoplanin monoclonal antibody PMab-269 using alanine-scanning mutagenesis and ELISA. Monoclon Antib Immunodiagn Immunother 2021;40:196–200.
35. Beckmen KB, Keogh MJ, Burek-Huntington KA, Ylitalo GM, Fadely BS, and Pitcher KW: Organochlorine contaminant concentrations in multiple tissues of free-ranging Steller sea lions (*Eumetopias jubatus*) in Alaska. Sci Total Environ 2016;542:441–452.
36. Peñín I, Levin M, Acevedo-Whitehouse K, Jasperse L, Gebhard E, Gulland FMD, and De Guise S: Effects of polychlorinated biphenyls (PCB) on California sea lion (*Zalophus californianus*) lymphocyte functions upon in vitro exposure. Environ Res 2018;167:708–717.
37. Keogh MJ, Taras B, Beckmen KB, Burek-Huntington KA, Ylitalo GM, Fadely BS, Rea LD, and Pitcher KW: Organochlorine contaminant concentrations in blubber of young Steller sea lion (*Eumetopias jubatus*) are influenced by region, age, sex, and lipid stores. Sci Total Environ 2020; 698:134183.
38. Vos JG, Dybing E, Greim HA, Ladefoged O, Lambré C, Tarazona JV, Brandt I, and Vethaak AD: Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 2000; 30:71–133.
39. Ross P, De Swart R, Addison R, Van Loveren H, Vos J, and Osterhaus A: Contaminant-induced immunotoxicity in harbour seals: Wildlife at risk? Toxicology 1996;112:157–169.
40. Wang J, Hülck K, Hong SM, Atkinson S, and Li QX: Accumulation and maternal transfer of polychlorinated biphenyls in Steller Sea Lions (*Eumetopias jubatus*) from Prince William Sound and the Bering Sea, Alaska. Environ Pollut 2011;159:71–77.

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