MONOCLONAL ANTIBODIES IN IMMUNODIAGNOSIS AND IMMUNOTHERAPY
Volume 39, Number 3, 2020

DOI: 10.1089/mab.2020.0008

Detection of Lion Podoplanin Using the Antitiger Podoplanin Monoclonal Antibody PMab-231

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Monoclonal antibodies (mAbs) that specifically target podoplanin (PDPN), a marker for type I alveolar cells, are needed 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, and sheep PDPNs. However, no antilion PDPN (lioPDPN) antibody has been developed. In this study, possible cross-reaction between available anti-PDPN mAbs and lioPDPN was examined. Immunohistochemical analysis showed that antitiger PDPN mAb PMab-231 (IgG_{2a}, kappa) reacted with type I alveolar cells from lion lung, indicating that PMab-231 is useful for the detection of lioPDPN.

Keywords: lion podoplanin, PDPN, lioPDPN, PMab-231

Introduction

Podoplanin (PDPN) is a type I transmembrane sialoglycoprotein that induces platelet aggregation through platelet C-type lectin-like receptor-2 (CLEC-2).[1] It is composed of three platelet aggregation-stimulating (PLAG) domains, termed PLAG1–3 (EDxxVTPG sequence).[2] Previously, PLAG3 is the most important domain for platelet aggregation by human PDPN.[2–4] Recently, a PLAG-like domain (PLD; EDxxT sequence) was reported to be another critical sequence for PDPN–CLEC-2 interaction.[5]

Anti-PDPN monoclonal antibodies (mAbs) are useful in distinguishing lymphatic from vascular endothelial cells or type I from type II alveolar cells of the lung.[2,6] PDPNs from many animals have been characterized using specific anti-PDPN mAbs, including antimouse PDPN (PMab-1),[7] antirat PDPN (PMab-2),[8] antirabbit PDPN (PMab-32),[9] antidog PDPN (PMab-38[10] and PMab-48,[11]) antibovine PDPN (PMab-44),[12] anticat PDPN (PMab-52),[13] antipig PDPN (PMab-213),[14] anti-Tasmanian devil PDPN (PMab-233),[15] antialpaca PDPN (PMab-225),[16] antitiger PDPN (PMab-231),[17] antiwhale PDPN (PMab-237),[18] antigoat PDPN (PMab-235),[19] antihorse PDPN (PMab-219),[20] antibear PDPN (PMab-247),[21] and antisheep PDPN (PMab-256).[22] Furthermore, many antihuman PDPN mAbs have been developed, including NZ-1.2,[7] LpMab-3,[23] LpMab-7,[23,24] LpMab-9,[23] LpMab-10,[25] LpMab-12,[26] LpMab-13,[27] LpMab-17,[28] LpMab-19,[29] and LpMab-21.[30,31] LpMab-3, LpMab-9, LpMab-12, LpMab-19, and LpMab-21 are antiglycopeptide mAbs (GpMabs), epitopes of which include both peptides and glycans. Cancer-specific mAbs (CasMabs), such as LpMab-2[23,32] and LpMab-23,[33,34] may be used for PDPN-targeted therapy[23,32–35] since PDPN is expressed in many healthy tissues.

To date, antilion PDPN (lioPDPN) mAbs have not been reported. In the present study, cross-reaction between anti-PDPN mAbs for various species and lioPDPN were examined using immunohistochemical analyses.

Materials and Methods

Immunohistochemical analyses

Lion lungs were collected at necropsy at the University of Tokyo, fixed in 10% neutral-buffered formalin, and processed routinely to produce formalin-fixed paraffin-embedded tissue (FFPE) sections. Four micrometers histological sections were autoclaved in citrate buffer (pH 6.0; Nichirei Biosciences, Inc., Tokyo, Japan) for 20 minutes. After blocking with SuperBlock T20 (PBS) Blocking Buffer (Thermo Fisher Scientific, Inc., Waltham, MA) for 15 minutes, sections were incubated with anti-PDPN mAbs (10 μg/mL) for 1 hour at room temperature and treated using Envision+ Kit (Agilent Technologies, Inc., Santa Clara, CA) for 30 minutes. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB; Agilent Technologies, Inc.) for 2 minutes, and finally, sections were counterstained

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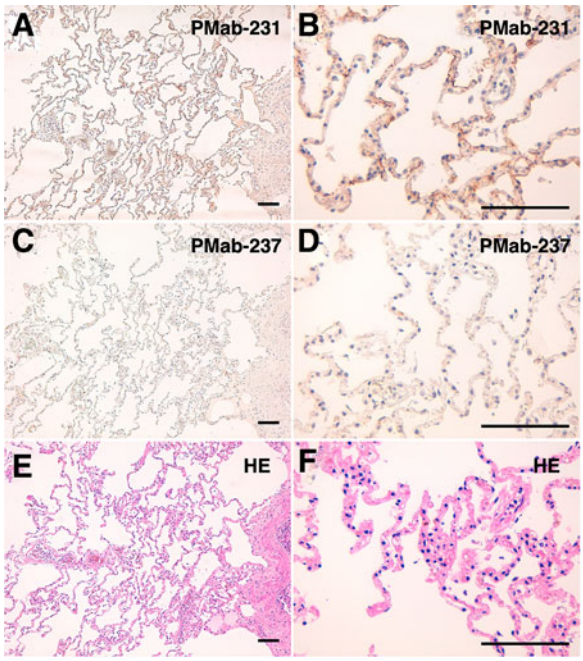

FIG. 1. Immunohistochemical analyses of lion lung tissues. Histological sections of the lion lung were directly autoclaved in citrate buffer for 20 minutes. After blocking, sections were incubated with 10 μg/mL of PMab-231 **(A, B)** or PMab-237 **(C, D)**, followed by detection using Envision+ Kit. **(E, F)** HE staining. Scale bar = 100 μm. HE, hematoxylin and eosin.

with hematoxylin/eosin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Results

In this study, cross-reaction between anti-PDPN mAbs against several mammalian species and lioPDPN was examined because a specific anti-lioPDPN mAb has not been available. Immunohistochemical analysis revealed that anti-tiger PDPN mAb PMab-231 (IgG_{2a}, kappa)[17] reacted with type I alveolar cells in lion lung (Fig. 1A, B). In contrast, PMab-237 (an antiwhale PDPN mAb) did not react with the lion lung using the next consecutive tissue section (Fig. 1C, D). Similarly, other anti-PDPN mAbs, including all mAbs already listed did not react with the lion lung using additional consecutive tissue sections (data not shown). Hematoxylin and eosin staining using the final consecutive tissue section indicated that tissue was well preserved (Fig. 1C).

Using the same methods, PMab-231 staining of other tissues was investigated, since PDPN is expressed in tissues other than lung.[6,36] PMab-231 did not stain renal podocytes, Bowman's capsule, or lymphatic endothelial cells (data not shown).

Discussion

To date, antilioPDPN mAbs have not been established due to lack of the relevant amino acid or DNA sequence of lioPDPN using fresh lion lung tissues. Instead, anti-PDPN mAbs developed for several mammalian tissues were tested for cross-reaction with lion lung, collected at necropsy, using immunohistochemical analyses. Only antitiger PDPN mAb (PMab-231) stained type I alveolar cells of lion lung (Fig. 1). Previously, PMab-231 specifically detected tiger PDPN using flow cytometry, Western blot, and immunohistochemical analyses.[17] PMab-231 cross-reacted with cat PDPN, and stained type I alveolar cells using immunohistochemistry. Dissociation constants (K_D) of PMab-231 for PDPN-expressing CHO-K1 cells (CHO/tigPDPN and cPDPN) were determined to be 1.2×10^{-8} and 1.9×10^{-8}, for tiger and cat, respectively, indicating similar affinity across species.

Although the staining intensity of PMab-231 against lion lung seems weak (Fig. 1), intensity of PMab-231 in cat lung was previously moderate to strong.[17] This difference is likely due to variation in binding epitopes between cat and lion, or PDPN protein in FFPE sections of lion tissues might have been damaged. Lack of reaction of PMab-231 with kidney and colon tissues remains unexplained. Similar data were observed previously using an antibovine PDPN mAb (PMab-44) against sheep,[37] goat,[38] and alpaca tissues.[39] Thus, expression levels of PDPN in the lung may be higher than levels in kidney or colon, or, perhaps binding epitopes of mAbs are different among tissues due to post-translational modification, such as glycosylation. Additional lion tissues could be investigated to further characterize immunoreactivity of PMab-231.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This research was supported, in part, by AMED under Grant Nos. JP20am0401013 (Y.K.), JP20am0101078 (Y.K.), and JP20ae0101028 (Y.K.), and by JSPS KAKENHI Grant No. 17K07299 (M.K.K.) and Grant No. 19K07705 (Y.K.).

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Received: March 9, 2020
Accepted: April 15, 2020