Podoplanin Expression in Canine Melanoma

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A type I transmembrane protein, podoplanin (PDPN), is expressed in several normal cells such as lymphatic endothelial cells or pulmonary type I alveolar cells. We recently demonstrated that anticanine PDPN monoclonal antibody (mAb), PMab-38, recognizes canine PDPN of squamous cell carcinomas, but does not react with lymphatic endothelial cells. Herein, we investigated whether PMab-38 reacts with canine melanoma. PMab-38 reacted with 90% of melanoma cells (9/10 cases) using immunohistochemistry. Of interest, PMab-38 stained the lymphatic endothelial cells and cancer-associated fibroblasts in melanoma tissues, although it did not stain any lymphatic endothelial cells in normal tissues. PMab-38 could be useful for uncovering the function of PDPN in canine melanomas.

Keywords: canine podoplanin, melanoma, monoclonal antibody, immunohistochemistry

A TYPE I TRANSMEMBRANE SIALOGLYCOPROTEIN, podoplanin (PDPN), is also known as gp40/T1 α /Aggrus.^(1–5) PDPN is expressed in normal cells including renal podocytes, pulmonary type I alveolar cells, and lymphatic endothelial cells.⁽⁴⁾ PDPN activates platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2) on platelets,^(6,7) and the PDPN–CLEC-2 interaction facilitates blood/lymphatic vessel separation.⁽⁸⁾ The expression of human PDPN was reported in many tumors, including oral cancers,⁽⁹⁾ malignant brain tumors,^(10–12) lung cancers,⁽¹³⁾ esophageal cancers,⁽¹⁴⁾ malignant mesotheliomas,⁽¹⁵⁾ testicular tumors,⁽¹⁶⁾ and osteosarcomas.⁽¹⁷⁾ PDPN expression is also associated with cancer metastasis and malignant progression.^(6,10)

We previously developed an anticanine PDPN monoclonal antibody (mAb), PMab-38,⁽¹⁸⁾ which is useful for immunohistochemistry (IHC), flow cytometry, and Western blotting. Recently, we demonstrated that PMab-38 can recognize PDPN of canine squamous cell carcinomas using IHC.⁽¹⁹⁾ Tumor cells in 15 out of 18 canine squamous cell carcinomas (83%) were stained by PMab-38 in IHC. Cancer-associated fibroblasts in 14 out of 18 cases (78%) were detected by PMab-38. In this study, we investigated whether canine melanoma was stained by PMab-38 because mouse PDPN expression and human PDPN expression were observed in melanomas.^(20,21) Watanabe et al. reported that high expression of mouse PDPN is associated with the metastatic ability in metastatic variants of B16 melanomas.⁽²⁰⁾

We stained 10 canine oral melanomas, which are the most frequent oral cancers in dog.⁽²²⁾ As depicted in Figures 1 and 2A, melanoma cells were stained by PMab-38 in 9 of 10 cases. Although melanoma cells were not stained by PMab-38 in case 9 (Fig. 1), cancer-associated fibroblasts were recognized by PMab-38 (Fig. 2B). Both melanoma cells and cancer-associated fibroblasts were stained in case 5 (Fig. 1). Kan et al. reported that PDPN expression in cancer-associated fibroblasts correlates with aggressive behavior in human melanoma,⁽²¹⁾ indicating that PDPN in cancer-associated fibroblasts of melanoma tissues might serve as a useful prognostic factor not only in human melanoma but also in canine melanoma. As shown in Figure 2C, PDPN of lymphatic endothelial cells was detected by PMab-38 in melanoma tissues, although lymphatic endothelial cells in normal tissues⁽¹⁸⁾ or squamous cell carcinomas⁽¹⁹⁾ were not stained by PMab-38, indicating that PDPN expression might be upregulated in melanoma, or post-translational modification of canine PDPN might be different between squamous cell carcinomas and melanomas.

We recently reported that the PMab-38 epitope is far from the platelet aggregation-stimulating domain of PDPN.⁽¹⁹⁾ However, we have not clarified whether the epitope of PMab-38 includes the post-translational modification. In the near future, we should determine the PMab-38 epitope using alanine scanning or glycan-deficient cells and uncover the difference of PDPN expression in several tissues.

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FIG. 1. Immunohistochemical analysis against canine melanoma using PMAb-38. Canine melanomas (10 cases) were obtained from North Lab (Hokkaido, Japan). Four micrometers thick histologic sections were deparaffinized in xylene, rehydrated, and autoclaved in citrate buffer (pH 6.0; Dako, Glostrup, Denmark) for 20 minutes. Sections were incubated with 10 μ g/mL of PMAb-38 overnight at 4°C followed by treatment with Envision+ kit for 30 minutes (Dako). As a control, blocking buffer was used in this study. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (Dako) for 2 minutes, after which the sections were counterstained with hematoxylin (Wako Pure Chemical Industries Ltd., Osaka, Japan). H&E staining was also performed. Scale bar: 100 μ m. mAb, monoclonal antibody; PDPN, podoplanin.

FIG. 2. PMAb-38 reacted with PDPN of melanoma cells, cancer-associated fibroblasts, and lymphatic endothelial cells. Sections of melanomas (A, case 7; B, case 9; C, case 2) were incubated with 10 μ g/mL of PMAb-38 overnight at 4°C followed by treatment with Envision+ kit for 30 minutes (Dako). As a control, blocking buffer was used. Color was developed using 3,3-diaminobenzidine tetrahydrochloride (Dako) for 2 minutes, after which the sections were counterstained with hematoxylin (Wako). H&E staining was also performed. Scale bar: 100 μ m. (A) staining of melanoma cells by PMAb-38, (B) staining of cancer-associated fibroblasts by PMAb-38, and (C) staining of lymphatic endothelial cells by PMAb-38.

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Author Disclosure Statement

No competing financial interests exist.

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