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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

TYPE I TRANSMEMBRANE SIALOGLYCOPROTEIN, podoplanin (PDPN), is also known as gp40/T1α/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.

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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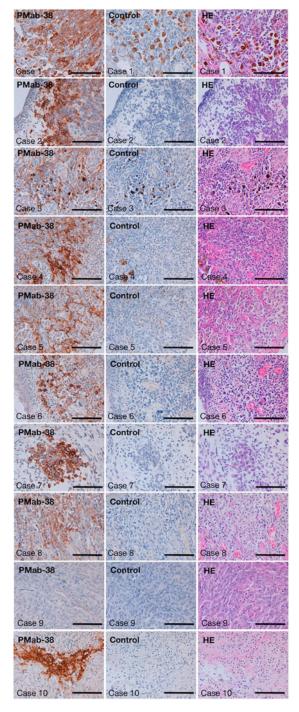


FIG. 1. Immunohistochemical analysis against canine melanoma using PMab-38. Canine melanomas (10 cases) were obtained from North Lab (Hokkaido, Japan). Four micrometers thick histologic sections were deparaffinized in xylene, rehydrated, and autoclaved in citrate buffer (pH 6.0; Dako, Glostrup, Denmark) for 20 minutes. Sections were incubated with 10 μg/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 μm. mAb, monoclonal antibody; PDPN, podoplanin.

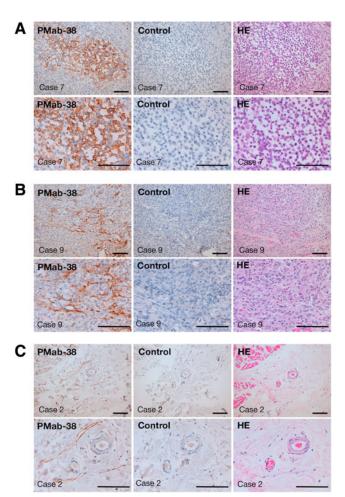


FIG. 2. PMab-38 reacted with PDPN of melanoma cells, cancer-associated fibroblasts, and lymphatic endothelial cells. Sections of melanomas (**A**, case 7; **B**, case 9; **C**, case 2) were incubated with 10 μg/mL of PMab-38 overnight at 4°C followed by treatment with Envision+ kit for 30 minutes (Dako). 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 μm. (**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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