PMab-38 Recognizes Canine Podoplanin of Squamous Cell Carcinomas

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Podoplanin, a type I transmembrane protein, is expressed in lymphatic endothelial cells. Although we previously developed an anticanine podoplanin monoclonal antibody (mAb), PMab-38, immunohistochemistry (IHC) showed that it did not react with canine lymphatic endothelial cells. Here, we determined whether PMab-38 recognizes canine podoplanin of squamous cell carcinomas (SCCs) and clarified its epitope. In IHC, PMab-38 reacted with 83% of SCCs (15/18 cases). Flow cytometry showed that the epitope of PMab-38 was different from that of the platelet aggregation-stimulating domain of the N-terminus, which was detected by almost all antipodoplanin mAbs such as D2–40 or NZ-1. PMab-38 is expected to be useful for investigating the function of podoplanin in canine tumors.

Keywords: canine podoplanin, monoclonal antibody, immunohistochemistry

Podoplanin is a type I transmembrane sialoglycoprotein, also known as Aggrus/T1a. Canine podoplanin was first reported as gp40. It is expressed in normal cells including renal podocytes, lymphatic endothelial cells, and pulmonary type I alveolar cells. Podoplanin activates platelet aggregation by binding to C-type lectin-like receptor-2 (CLEC-2) on platelets, and the interaction between podoplanin and CLEC-2 facilitates blood/lymphatic vessel separation. The expression of human podoplanin has been reported in many malignant tumors, such as oral squamous cell carcinomas (SCCs), malignant brain tumors, lung cancers, esophageal cancers, malignant mesotheliomas, testicular tumors, and osteosarcomas. Podoplanin expression is also associated with malignant progression and cancer metastasis. However, canine podoplanin in tumors has not been investigated because of the absence of a specific and sensitive monoclonal antibody (mAb). We recently reported anticanine podoplanin mAb, PMab-38, which is useful for immunohistochemistry (IHC). Furthermore, PMab-38 specifically reacts with canine podoplanin in flow cytometry and Western blotting.

Here, we determined whether PMab-38 can recognize podoplanin of canine SCCs because human podoplanin is highly expressed in these cells and is involved in tumor malignancy. We first checked the PMab-38 reactivity against SCCs (8 oral SCCs and 10 skin SCCs) in IHC. Among them, only case 16 (1/18; 5.6%) was detected by PMab-38 in >50% of tumor cells. In total, tumor cells in 15 out of 18 cases (83%) were stained with PMab-38 in a membrane-staining pattern. Similarly, cancer-associated fibroblasts in 14 out of 18 cases (78%) were detected by PMab-38. Human podoplanin expression in several cancers contributes to poor prognosis; therefore, PMab-38 might be useful for investigating the pathological function of canine podoplanin in the tumor microenvironment.

Next, we investigated the epitope of PMab-38 using flow cytometry. To this end, we produced several deletion mutants of canine podoplanin, which are expressed in CHO-K1 cells, including dN23 (corresponding to 23–169 amino acids [aa]), dN40 (corresponding to 40–169 aa), dN60 (corresponding to 60–169 aa), dN80 (corresponding to 80–169 aa), and dN100 (corresponding to 100–169 aa). Antipodoplanin mAbs usually detect the platelet aggregation-inducing (PLAG) domain, particularly PLAG1–PLAG3 (corresponding to 36–61 aa) near the N-terminus; in contrast, PMab-38 reacted with dN23, dN40, dN60, and dN80. When antipodoplanin mAbs reacted with the PLAG domain, they could not react with dN60, dN80, and dN100, indicating that the PMab-38 epitope is far from PLAG1–PLAG3 (Fig. 2). Previously, we
Immunohistochemical analysis against canine SCCs by PMab-38. Canine tissues (A, oral SCCs; B, skin SCCs) were obtained from North Lab (Hokkaido, Japan). In total, 4 μm 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). Color was developed using 3, 3-diaminobenzidine tetrahydrochloride (DAB; Dako) for 2 minutes, after which the sections were counterstained with hematoxylin (Wako Pure Chemical Industries Ltd., Osaka, Japan). Hematoxylin and eosin (HE) staining was also performed. Scale bar: 100 μm.
produced an antihuman podoplanin mAb, LpMab-7, the epitope of which is Arg79-Leu83 of human podoplanin, which corresponds to His88-Gly90 of canine podoplanin. LpMab-7 demonstrated high sensitivity compared with other antihuman podoplanin mAbs, suggesting that mAbs against these epitopes have high sensitivity and specificity against not only human podoplanin but also canine podoplanin.

Taken together, these data show that PMab-38 could be useful for uncovering the pathophysiological function of podoplanin in canine tumors. PMab-38 did not react with the lymphatic endothelium in our previous study; therefore, the PMab-38 epitope might be involved in cancer specificity of canine podoplanin.

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