The Journal of Veterinary Medical Science

NOTE

Surgery

Podoplanin promotes cell proliferation, survival, and migration of canine non-tonsillar squamous cell carcinoma

Masahiro SHINADA[1)], Daiki KATO[1)]*, Masaya TSUBOI[2)], Namiko IKEDA[1)], Susumu AOKI[1)], Takaaki IGUCHI[1)], Toshio LI[1)], Yuka KODERA[1)], Ryosuke OTA[1)], Shoma KOSEKI[1)], Hayato SHIBAHARA[1)], Yosuke TAKAHASHI[2)], Yuko HASHIMOTO[2)], James K CHAMBERS[3)], Kazuyuki UCHIDA[3)], Shunsuke NOGUCHI[4)], Yukinari KATO[5,6)], Ryohei NISHIMURA[1)], Takayuki NAKAGAWA[1)]

[1)]Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[2)]Veterinary Medical Center, The University of Tokyo, Tokyo, Japan
[3)]Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[4)]Laboratory of Veterinary Radiology, Graduate School of Veterinary Science, Osaka Metropolitan University, Osaka, Japan
[5)]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Miyagi, Japan
[6)]Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Miyagi, Japan

J. Vet. Med. Sci.
85(10): 1068–1073, 2023
doi: 10.1292/jvms.23-0062

Received: 7 February 2023
Accepted: 26 July 2023
Advanced Epub:
4 August 2023

ABSTRACT. Podoplanin (PDPN) is a prognostic factor and is involved in several mechanisms of tumor progression in human squamous cell carcinoma (SCC). Canine non-tonsillar SCC (NTSCC) is a common oral tumor in dogs and has a highly invasive characteristic. In this study, we investigated the function of PDPN in canine NTSCC. In canine NTSCC clinical samples, PDPN overexpression was observed in 80% of dogs with NTSCC, and PDPN expression was related to ki67 expression. In PDPN knocked-out canine NTSCC cells, cell proliferation, cancer stemness, and migration were suppressed. As the mechanism of PDPN-mediated cell proliferation, PDPN knocked-out induced apoptosis and G2/M cell cycle arrest in canine NTSCC cells. These findings suggest that PDPN promotes tumor malignancies and may be a novel biomarker and therapeutic target for canine NTSCC.

KEYWORDS: canine squamous cell carcinoma, ki67, migration, podoplanin, proliferation

Podoplanin (PDPN), also known as PA2.26, gp38, T1a, and Aggrus is the type 1 transmembrane glycoprotein [22, 33] and is expressed in various normal cell types such as renal podocytes, pulmonary type I alveolar cells, and lymphatic endothelial cells (LECs) [22, 33]. In the embryo, PDPN plays a crucial role in development of some organs [33]. In adults, PDPN promotes lymphangiogenesis, maintains high endothelial venule integrity, and regulates immune systems [12, 22, 25, 33]. PDPN has been reported to be overexpressed in several types of humans, canine and feline tumors including squamous cell carcinoma (SCC) [16, 17, 20, 21, 33]. In human SCC, high PDPN expression was related to a frequency of tumor metastasis and poor prognosis of patients with SCC [6, 11, 20, 38]. Previous experimental results showed that PDPN was related to cell survival, migration, and invasion of human SCC cells *in vitro* and *in vivo* [23, 27, 28, 34]. In addition, it was reported that anti-PDPN antibody exhibited anti-tumor effects on human SCC cell growth in mouse xenograft models [18]. Therefore, PDPN promotes tumor malignancy and can be a new therapeutic target for human SCC.

Canine oral SCC is the second most common epithelial malignancy of the canine oral cavity [29]. Canine oral SCC develops predominantly in gingiva (non-tonsillar SCC; NTSCC) and tonsil, and more than a half of oral SCC were NTSCC [15, 29]. Surgical resection is the first line of treatment for canine NTSCC, because lymph node and distant metastasis is not frequent [29]. However, canine NTSCC is locally aggressive with local bone invasion seen in 77% of cases, and complete tumor cell resection is challenging when the tumor was advanced and aggressively invaded local bones [36]. Therefore, it is important to reveal the mechanism of the canine NTSCC progression. Recently, we have revealed that canine NTSCC expresses PDPN in tumor cells [35]. This finding gives

*Correspondence to: Kato D: adk@g.ecc.u-tokyo.ac.jp, Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

us a possible hypothesis that PDPN expressed in canine NTSCC promotes tumor malignancy and may be a therapeutic target as well as a diagnostic and prognostic marker. The aim of this study was to investigate the role of PDPN in canine NTSCC.

The expression of PDPN in canine NTSCC was compared with that of normal canine squamous epithelial cells, and relationship between PDPN expression and ki67 expression in canine NTSCC tissues was evaluated by immunohistochemistry. Further, to evaluate the functions of PDPN in NTSCC cells, a PDPN knocked-out (PDPN-KO) canine NTSCC cell line, oSCC3 [32], was generated and changes of cell migration, cancer stemness, cell proliferation, apoptosis, and cell cycle were analyzed. Detailed materials and methods are described in a Supplementary file.

PDPN expression on epithelial cells of normal skin (n=4), esophagus (n=4), and tumor cells of NTSCC (n=10) was evaluated by immunohistochemistry. Normal lung tissue was used as a positive control for PDPN staining. All NTSCC tissues were derived from the oral cavity and the primary tumor sites were as follows; mandible (n=6), buccal mucosa (n=2), maxilla (n=1), and pharynx and larynx (n=1). Although no expression of PDPN was detected in normal skin and esophageal tissues, 80% (8/10) of NTSCC tissues expressed PDPN in tumor cells (Fig. 1A). The expression of PDPN in positively stained samples was strong in the cell membranes of tumor cells and moderate in the cytoplasm of tumor cells, which was similar to the expression pattern observed in the positive control canine lung tissues (Fig. 1A). The proportion of positively stained tumor cells was varied among tumor samples (mean: 48.5%, range: 0–100%). Next, we investigated the relationship between PDPN expression and ki67 expression, which is the biomarker for

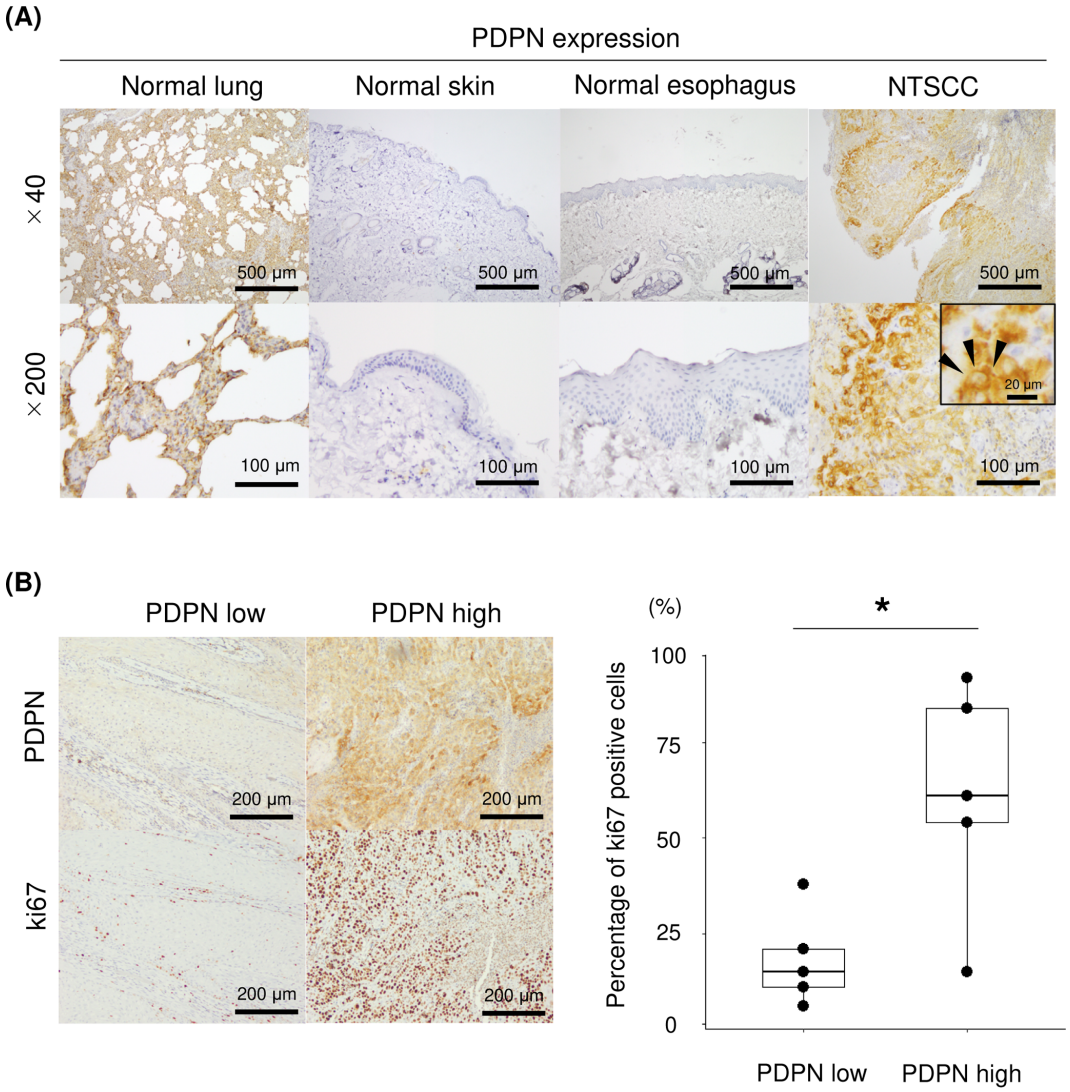

Fig. 1. Podoplanin (PDPN) expression in canine normal and non-tonsillar squamous cell carcinoma (NTSCC) tissues and a relationship between PDPN and ki67 expression in canine NTSCC tissues. (**A**) Representative images of PDPN staining for canine normal lung (a positive control of staining for PDPN), skin, esophagus, and NTSCC tissues. An enlarged image is shown. Black arrowheads indicate PDPN expression in cellular membranes. (**B**) Left panel: representative images of PDPN and Ki67 expression in NTSCC tissues. Right panel: The percentage of Ki67-positive tumor cells in canine NTSCC tissues was compared between the PDPN high-expression group and the PDPN low-expression group. Box plots where box limits show 25th and 75th percentiles, the horizontal line shows the median, and dot plots in boxplots indicate a percentage of each tissue. Welch's *t* test, *: $P<0.05$.

lymph node metastasis characteristics of canine oral SCC [26]. Ten NTSCC tissues were divided in PDPN high expression or low expression according to the median percentage (42%) of PDPN positive tumor cells. The percentage of ki67 positive tumor cells in the tissues with high PDPN expression was significantly higher than that in the tissues with low PDPN expression (Fig. 1B). This result suggested that PDPN was related to proliferation of canine NTSCC cells.

To investigate the functions of PDPN in canine NTSCC cells, PDPN-KO canine NTSCC cells were generated using CRISPR/Cas9 technology from the primary canine NTSCC cell line: oSCC3. The single guide RNA targeting the canine PDPN gene depleted the PDPN protein expression on the cell surface (Fig. 2A) with confirmation of genetic mutations in PDPN genes in the canine NTSCC cell line (Fig. 2B). Cell proliferation of PDPN-KO canine NTSCC cells was strongly suppressed, and the number of tumor cells was reduced to one-fourth compared to normal oSCC3 cells (control cells) (Fig. 2C). In addition, PDPN-KO canine NTSCC cells also exhibited reduced sphere-forming ability (Supplementary Fig. 1A) and a lower migratory ability in wound-healing assay compared to control cells (Supplementary Fig. 1B). These results indicated that PDPN regulated cell proliferation, cancer stemness, and cell migration in canine NTSCC cells.

To investigate the mechanism of PDPN-KO related suppression of cell proliferation in canine NTSCC cells, induction of apoptosis and changes of cell cycle were evaluated in PDPN-KO canine NTSCC cells. As a result, both early and late apoptotic cells were significantly increased in PDPN-KO canine NTSCC cells compared to those in control cells (Fig. 3A and 3B). In addition, expression of cleaved caspase 3, an indicator for initiation of apoptosis, was also upregulated in PDPN-KO canine NTSCC cells. Moreover, expression of Bax, a pro-apoptotic protein, was upregulated and expression of Bcl-2 and Bcl-xL, anti-apoptotic proteins, were downregulated in PDPN-KO canine NTSCC cells, compared to control cells by western blot analysis (Fig. 3C). In cell cycle analysis, the proportion of G2/M-phase cells was significantly increased and the proportion of G0/G1-phase cells was significantly decreased in PDPN-KO canine NTSCC cells compared to control cells, indicating the induction of G2/M cell cycle arrest (Fig. 3D). The expression of G2/M cell cycle related proteins was evaluated by western blot analysis and the expression of phospho-chk2 (Thr68), phospho-cdc2 (Tyr15), p53, and p27 was upregulated, and cyclin B1 expression was downregulated in PDPN-KO canine NTSCC cells compared to control cells (Fig. 3E). Overall, these findings suggested that the suppression of cell proliferation observed in PDPN depleted canine NTSCC cells would be caused by the induction of apoptosis and G2/M cell cycle arrest through the alteration of G2/M related proteins.

In this study, we first demonstrated the association between the PDPN expression and the ki67 expression in the NTSCC clinical tissue samples. Moreover, the abrogation of PDPN in canine NTSCC cells suppressed tumor cell proliferation. In addition, PDPN-KO canine NTSCC cells showed induction of both apoptosis and G2/M cell cycle arrest. These results suggested that PDPN is related to tumor malignancies of canine NTSCC through the several mechanisms.

We revealed that PDPN expression was related to ki67 expression in canine NTSCC tissues. Ki67 is one of the representative proliferation markers, and ki67 is used as a prognostic marker in various tumors such as canine melanoma and human breast cancer [4, 8]. Therefore, PDPN would be related to canine NTSCC progression by promoting tumor cell proliferation. In addition, ki67 is

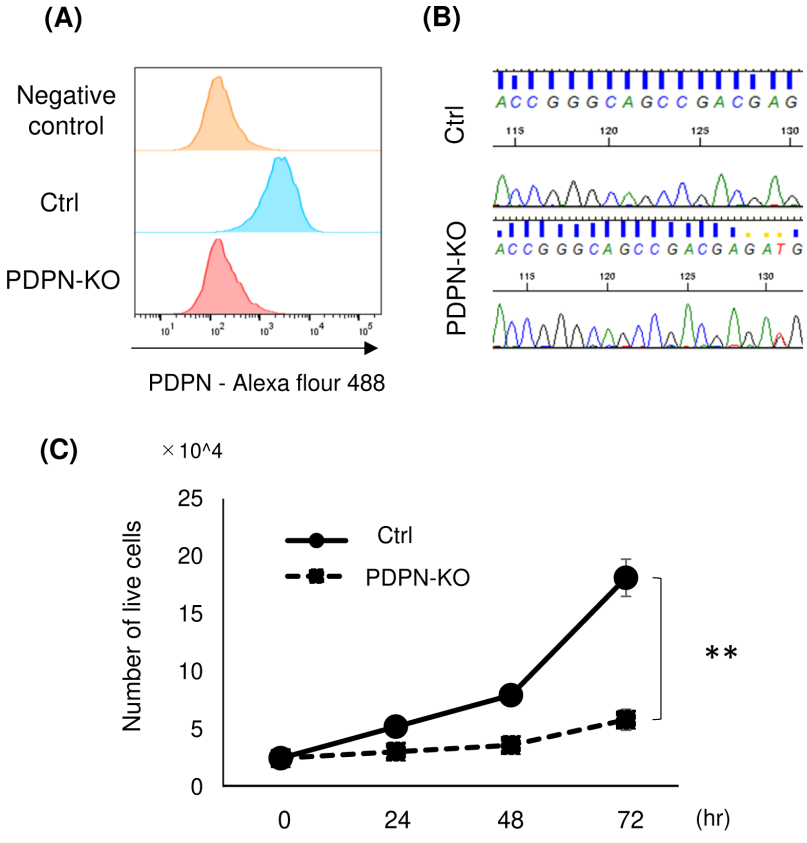

Fig. 2. Investigating the association between podoplanin (PDPN) and tumor malignancy in canine non-tonsillar squamous cell carcinoma (NTSCC) cells. (**A**) PDPN expression of control and podoplanin knocked-out (PDPN-KO) oSCC3 cells. PDPN expression was detected by flowcytometry. (**B**) Base sequences of control and PDPN-KO oSCC3 cells. The region targeted by single guide RNA is shown. (**C**) The number of live cells of control and PDPN-KO oSCC3 cells after 24, 48, and 72 hr of cell seeding (n=3). Representative images are shown under the graph. All experiments were performed in triplicate. Bar graphs are indicated as the mean ± SD. Welch's *t* test, **: $P<0.01$.

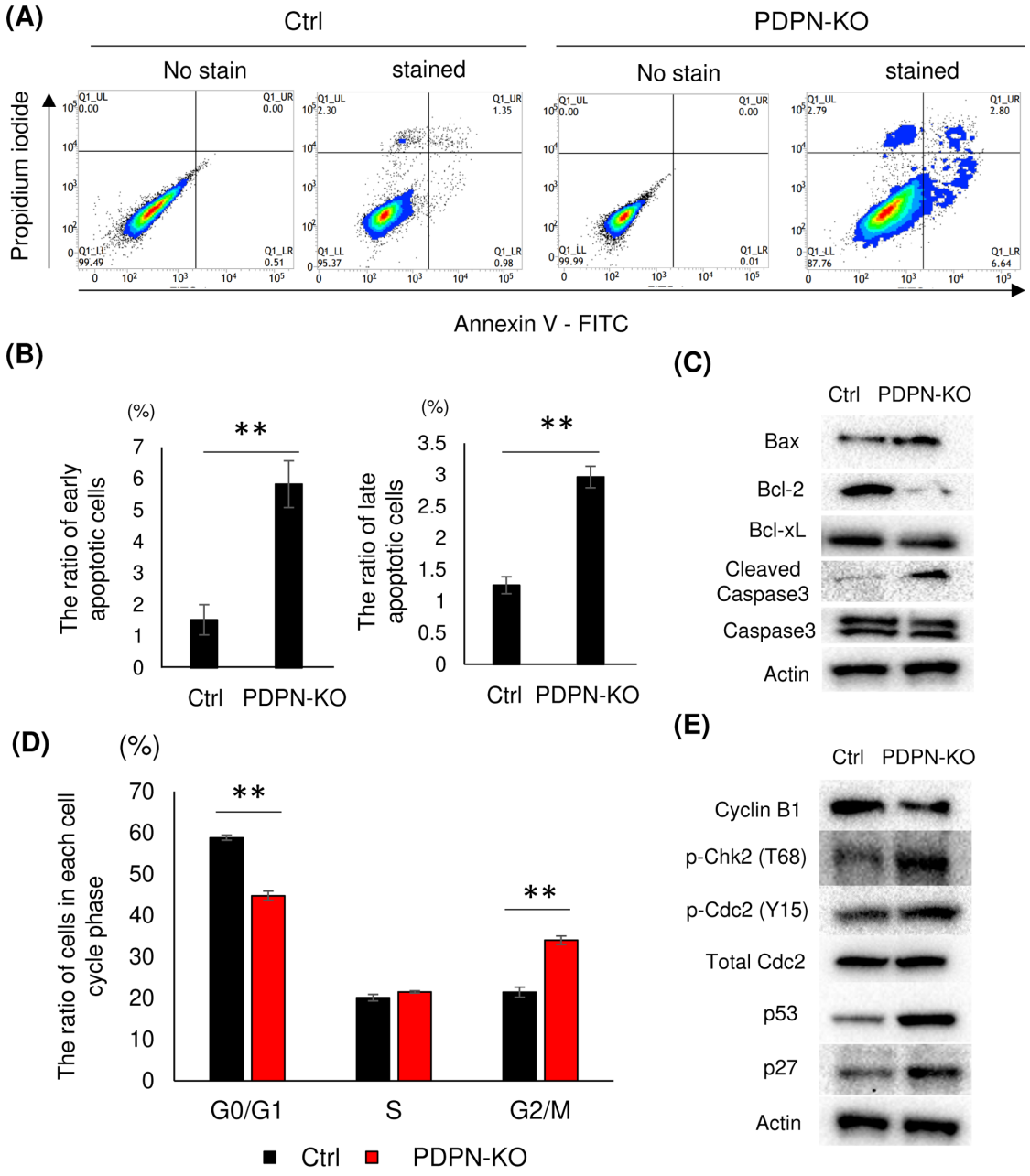

Fig. 3. Effects of podoplanin knocked-out (PDPN-KO) on apoptosis and cell cycle of canine non-tonsillar squamous cell carcinoma (NTSCC) cells. (**A**) Annexin V-FITC/propidium iodide analysis of apoptosis in control and PDPN-KO oSCC3 cells. The lower-right panel presents early apoptotic cells, and the upper-right panel shows late apoptotic cells. Non-stained cells were used as a control (n=3). (**B**) The percentage of early apoptotic cells (left panel) and late apoptotic cells (right panel) in control and PDPN-KO oSCC3 cells (n=3). (**C**) Apoptotic related protein expression of control and PDPN-KO oSCC3 cells detected by western blot. (**D**) Proportions of each cell cycle phase of control and PDPN-KO oSCC3 cells (n=3). (**E**) G2/M cell cycle-related protein expression of control and PDPN-KO oSCC3 cells detected by western blot. All experiments were performed in triplicate. Bar graphs are indicated as the mean ± SD. Welch's *t* test, **: $P<0.01$.

reported to be the predictive marker of lymph node metastasis in canine NTSCC [26]. Therefore, PDPN expression might be a poor prognostic factor of canine NTSCC, similar to findings in medicine that PDPN expression was associated with the frequency of lymph node metastasis and poor prognosis in human SCC [6, 11, 20, 38]. We could not find the relationship between PDPN expression and clinicopathological features of dogs with NTSCC due to a small sample size and further large cohort investigation would give us significant clinical value of PDPN expression in canine NTSCC.

In PDPN-KO canine NTSCC cells, cell proliferation was suppressed through the induction of apoptosis and G2/M cell cycle arrest. Previous reports indicated that PDPN promoted cell proliferation in human SCC cells and canine melanoma cells [14, 35], but the regulating mechanism of PDPN in tumor cell proliferation had not been elucidated. In this study, p27 expression was also

upregulated by PDPN-KO. Previous studies showed that p27 expression was upregulated by p53 and an accumulation of p27 induced G2/M cell cycle arrest [13, 30]. Moreover, phosphor-chk2, phosphor-cdc2, and p53 expression were upregulated by PDPN-KO. One of the major regulators of the chk/p53 cascade is ataxia telangiectasia (ATM), a DNA damage sensor [3, 19]. When DNA damage occurs, the chk/p53 cascade is activated by ATM, and the activated chk/p53 cascade suppresses the activation of cyclin B1 and cdc2 complex, which induces G2/M cell cycle arrest [3, 19]. Therefore, it was possible that G2/M cell cycle arrest induced by PDPN-KO was depended on the activation of the chk/p53 cascade. Other possible mechanisms of inhibition of tumor cell proliferation induced by PDPN-KO are NF-kB pathways and Rho associated kinase (ROCK) signaling. In the LECs, PDPN promotes lymphatic growth through mitogen-activated protein kinase and NF-kB pathways [25]. In lymph node stroma, PDPN regulates lymph node expansion through Rho associated kinase (ROCK) signaling [1]. It was also reported that PDPN promoted human SCC cell invasion and the growth of human mesothelioma cells through ROCK signaling [24, 37]. Since these pathways were reported to regulate apoptosis and/or G2/M cell cycle arrest in human tumor cells [5, 7, 31, 40, 41]. These pathways might also regulate PDPN-related cell proliferation in canine NTSCC cells. Identification of the specific signaling pathway regulating PDPN-mediated cell proliferation is desired and it would allow PDPN to be used as a novel therapeutic target in canine NTSCC.

PDPN-KO suppressed the migration of canine NTSCC cells in wound-healing assay. Canine NTSCC has locally aggressive clinical characteristics, and approximately 80% of canine NTSCC invaded local bones [36]. In addition, up to 36% of NTSCC showed distant metastasis [9]. PDPN has been reported to promote tumor cell migration and invasion through various mechanisms in human SCC. One reported mechanism was that PDPN activated Rho-GTPase family proteins to remodel cellular cytoskeleton into a suitable form of cell migration [24]. Another report showed that PDPN regulated epithelial-mesenchymal transition to acquire highly migrative and invasive capabilities [39]. The detailed mechanism of PDPN promoting migration in canine NTSCC cells needs to be investigated in further study and PDPN promoting migration might be involved in aggressive local invasion and metastatic characteristics of canine NTSCC.

PDPN-KO canine NTSCC cells also showed lower sphere forming capacity compared to control cells, indicating that PDPN was related to cancer stemness of canine NTSCC cells. Previous reports suggested that PDPN is one of the cancer stem cell markers of human SCC, and PDPN enhanced cancer stemness to support cell survival [27]. Cancer stem cells are subpopulations of tumor cells which have highly resistant capacities to chemotherapy and radiotherapy [2]. Although canine NTSCC is responsive to radiotherapy, 16% and 53% of dogs showed local recurrence and tumor regrowth, respectively, even after adjuvant radiotherapy [9, 10]. Furthermore, canine NTSCC is often unresponsive to systemic chemotherapy [36]. Therefore, it is possible that PDPN is also related to resistance to chemotherapy and radiotherapy by regulating cancer stemness in canine NTSCC cells.

In this study, we performed experiments with only one PDPN-KO cell line, thus; an off-target effect of CRISPR/Cas9 technology could not denied completely. To show the function of the PDPN more clearly, further studies were required.

Overall, we found the association between PDPN expression and proliferative marker expression in canine NTSCC and the novel malignant mechanisms of PDPN in canine NTSCC cells which can explain the aggressive clinical behaviors of canine NTSCC. Our results suggested the potential of PDPN as a novel prognostic and diagnostic marker and therapeutic target.

CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. We thank all the clinicians who treated the dogs, pathologists who processed the samples, and owners of the dogs. This research was supported in part by JSPS KAKENHI under Grant Number: 19K22361 and 21K20614, and by Japan Agency for Medical Research and Development (AMED) under Grant Numbers: JP22ama121008 and JP22am0401013.

REFERENCES

1. Acton SE, Farrugia AJ, Astarita JL, Mourão-Sá D, Jenkins RP, Nye E, Hooper S, van Blijswijk J, Rogers NC, Snelgrove KJ, Rosewell I, Moita LF, Stamp G, Turley SJ, Sahai E, Reis e Sousa C. 2014. Dendritic cells control fibroblastic reticular network tension and lymph node expansion. *Nature* **514**: 498–502. [Medline] [CrossRef]
2. Atashzar MR, Baharlou R, Karami J, Abdollahi H, Rezaei R, Pourramezan F, Zoljalali Moghaddam SH. 2020. Cancer stem cells: a review from origin to therapeutic implications. *J Cell Physiol* **235**: 790–803. [Medline] [CrossRef]
3. Awasthi P, Foiani M, Kumar A. 2015. ATM and ATR signaling at a glance. *J Cell Sci* **128**: 4255–4262. [Medline]
4. Bergin IL, Smedley RC, Esplin DG, Spangler WL, Kiupel M. 2011. Prognostic evaluation of Ki67 threshold value in canine oral melanoma. *Vet Pathol* **48**: 41–53. [Medline] [CrossRef]
5. Berra E, Diaz-Meco MT, Moscat J. 1998. The activation of p38 and apoptosis by the inhibition of Erk is antagonized by the phosphoinositide 3-kinase/Akt pathway. *J Biol Chem* **273**: 10792–10797. [Medline] [CrossRef]
6. Cañueto J, Cardeñoso-Álvarez E, Cosano-Quero A, Santos-Briz Á, Fernández-López E, Pérez-Losada J, Román-Curto C. 2017. The expression of podoplanin is associated with poor outcome in cutaneous squamous cell carcinoma. *J Cutan Pathol* **44**: 144–151. [Medline] [CrossRef]
7. Cheng C, Seen D, Zheng C, Zeng R, Li E. 2021. Role of small GTPase RhoA in DNA damage response. *Biomolecules* **11**: 212. [Medline] [CrossRef]
8. de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ, Paesmans M. 2007. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. *Br J Cancer* **96**: 1504–1513. [Medline] [CrossRef]
9. Gillette EL, McChesney SL, Dewhirst MW, Scott RJ. 1987. Response of canine oral carcinomas to heat and radiation. *Int J Radiat Oncol Biol Phys* **13**: 1861–1867. [Medline] [CrossRef]
10. Grier CK, Mayer MN. 2007. Radiation therapy of canine nontonsillar squamous cell carcinoma. *Can Vet J* **48**: 1189–1191. [Medline]
11. Hamada M, Ebihara Y, Nagata K, Yano M, Kogashiwa Y, Nakahira M, Sugasawa M, Nagatsuka H, Yasuda M. 2020. Podoplanin is an efficient

predictor of neck lymph node metastasis in tongue squamous cell carcinoma with low tumor budding grade. *Oncol Lett* **19**: 2602–2608. [Medline]
12. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, Pan Y, Sheng M, Yago T, Silasi-Mansat R, McGee S, May F, Nieswandt B, Morris AJ, Lupu F, Coughlin SR, McEver RP, Chen H, Kahn ML, Xia L. 2013. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature* **502**: 105–109. [Medline] [CrossRef]
13. Hsu SP, Lin PH, Chou CM, Lee WS. 2019. Progesterone up-regulates p27 through an increased binding of the progesterone receptor-A-p53 protein complex onto the non-canonical p53 binding motif in HUVEC. *J Steroid Biochem Mol Biol* **185**: 163–171. [Medline] [CrossRef]
14. Hsu YB, Huang CF, Lin KT, Kuo YL, Lan MC, Lan MY. 2019. Podoplanin, a potential therapeutic target for nasopharyngeal carcinoma. *BioMed Res Int* **2019**: 7457013. [Medline] [CrossRef]
15. Jang HS, Kim JI, Kim JH, Jang KH. 2004. Combined therapy with carboplatin and meloxicam for oral squamous cell carcinoma in a dog. *J Am Vet Med Assoc* **224**: 388–394. [CrossRef]
16. Kamoto S, Shinada M, Kato D, Yoshimoto S, Ikeda N, Tsuboi M, Yoshitake R, Eto S, Hashimoto Y, Takahashi Y, Chambers J, Uchida K, Kaneko MK, Fujita N, Nishimura R, Kato Y, Nakagawa T. 2020. Phase I/II clinical trial of the anti-podoplanin monoclonal antibody therapy in dogs with malignant melanoma. *Cells* **9**: 2529. [Medline] [CrossRef]
17. Kamoto S, Shinada M, Kato D, Tsuboi M, Yoshimoto S, Yoshitake R, Eto S, Ikeda N, Takahashi Y, Hashimoto Y, Chambers J, Uchida K, Yamada S, Kaneko MK, Nishimura R, Kato Y, Nakagawa T. 2021. Expression of podoplanin in various types of feline tumor tissues. *J Vet Med Sci* **83**: 1795–1799. [Medline] [CrossRef]
18. Kato Y, Kunita A, Abe S, Ogasawara S, Fujii Y, Oki H, Fukayama M, Nishioka Y, Kaneko MK. 2015. The chimeric antibody chLpMab-7 targeting human podoplanin suppresses pulmonary metastasis via ADCC and CDC rather than via its neutralizing activity. *Oncotarget* **6**: 36003–36018. [Medline] [CrossRef]
19. Khanna KK, Lavin MF, Jackson SP, Mulhern TD. 2001. ATM, a central controller of cellular responses to DNA damage. *Cell Death Differ* **8**: 1052–1065. [Medline] [CrossRef]
20. Kim HY, Rha KS, Shim GA, Kim JH, Kim JM, Huang SM, Koo BS. 2015. Podoplanin is involved in the prognosis of head and neck squamous cell carcinoma through interaction with VEGF-C. *Oncol Rep* **34**: 833–842. [Medline] [CrossRef]
21. Kiname K, Yoshimoto S, Kato D, Tsuboi M, Tanaka Y, Yoshitake R, Eto S, Shinada M, Chambers J, Saeki K, Kinoshita R, Yamada S, Uchida K, Kaneko MK, Nishimura R, Kato Y, Nakagawa T. 2019. Evaluation of immunohistochemical staining with PMab-38, an anti-dog podoplanin monoclonal antibody, in various canine tumor tissues. *Jpn J Vet Res* **67**: 25–34.
22. Krishnan H, Rayes J, Miyashita T, Ishii G, Retzbach EP, Sheehan SA, Takemoto A, Chang YW, Yoneda K, Asai J, Jensen L, Chalise L, Natsume A, Goldberg GS. 2018. Podoplanin: An emerging cancer biomarker and therapeutic target. *Cancer Sci* **109**: 1292–1299. [Medline] [CrossRef]
23. Kunita A, Baeriswyl V, Meda C, Cabuy E, Takeshita K, Giraudo E, Wicki A, Fukayama M, Christofori G. 2018. Inflammatory Cytokines Induce Podoplanin Expression at the Tumor Invasive Front. *Am J Pathol* **188**: 1276–1288. [Medline] [CrossRef]
24. Martín-Villar E, Borda-d'Agua B, Carrasco-Ramirez P, Renart J, Parsons M, Quintanilla M, Jones GE. 2015. Podoplanin mediates ECM degradation by squamous carcinoma cells through control of invadopodia stability. *Oncogene* **34**: 4531–4544. [Medline] [CrossRef]
25. Maruyama Y, Maruyama K, Kato Y, Kajiya K, Moritoh S, Yamamoto K, Matsumoto Y, Sawane M, Kerjaschki D, Nakazawa T, Kinoshita S. 2014. The effect of podoplanin inhibition on lymphangiogenesis under pathological conditions. *Invest Ophthalmol Vis Sci* **55**: 4813–4822. [Medline] [CrossRef]
26. Mestrinho LA, Pissarra H, Carvalho S, Peleteiro MC, Gawor J, Niza MMRE. 2017. Comparison of histological and proliferation features of canine oral squamous cell carcinoma based on intraoral location: 36 cases. *J Vet Dent* **34**: 92–99. [Medline] [CrossRef]
27. Miyashita T, Higuchi Y, Kojima M, Ochiai A, Ishii G. 2017. Single cell time-lapse analysis reveals that podoplanin enhances cell survival and colony formation capacity of squamous cell carcinoma cells. *Sci Rep* **7**: 39971. [Medline] [CrossRef]
28. Miyata K, Takemoto A, Okumura S, Nishio M, Fujita N. 2017. Podoplanin enhances lung cancer cell growth in vivo by inducing platelet aggregation. *Sci Rep* **7**: 4059. [Medline] [CrossRef]
29. Mosca A, Gibson D, Mason SL, Dobson J, Giuliano A. 2021. A possible role of coarse fractionated radiotherapy in the management of gingival squamous cell carcinoma in dogs: A retrospective study of 21 cases from two referral centers in the UK. *J Vet Med Sci* **83**: 447–455. [Medline] [CrossRef]
30. Nakayama K, Nagahama H, Minamishima YA, Miyake S, Ishida N, Hatakeyama S, Kitagawa M, Iemura S, Natsume T, Nakayama KI. 2004. Skp2-mediated degradation of p27 regulates progression into mitosis. *Dev Cell* **6**: 661–672. [Medline] [CrossRef]
31. Neilsen BK, Frodyma DE, McCall JL, Fisher KW, Lewis RE. 2019. ERK-mediated TIMELESS expression suppresses G2/M arrest in colon cancer cells. *PLoS One* **14**: e0209224. [Medline] [CrossRef]
32. Noguchi S, Hattori A, Tanimoto N, Nishida R, Hirano K, Wada Y, Matsuyama S, Shimada T, Akiyoshi H. 2020. Establishing cell lines for canine tonsillar and non-tonsillar oral squamous cell carcinoma and identifying characteristics associated with malignancy. *Tissue Cell* **67**: 101408. [Medline] [CrossRef]
33. Quintanilla M, Montero-Montero L, Renart J, Martín-Villar E. 2019. Podoplanin in inflammation and cancer. *Int J Mol Sci* **20**: 707. [Medline] [CrossRef]
34. Schwab M, Lohr S, Schneider J, Kaiser M, Krunic D, Helbig D, Géraud C, Angel P. 2021. Podoplanin is required for tumor cell invasion in cutaneous squamous cell carcinoma. *Exp Dermatol* **30**: 1619–1630. [Medline] [CrossRef]
35. Shinada M, Kato D, Kamoto S, Yoshimoto S, Tsuboi M, Yoshitake R, Eto S, Ikeda N, Saeki K, Hashimoto Y, Takahashi Y, Chambers J, Uchida K, Kaneko MK, Fujita N, Nishimura R, Kato Y, Nakagawa T. 2020. PDPN is expressed in various types of canine tumors and its silencing induces apoptosis and cell cycle arrest in canine malignant melanoma. *Cells* **9**: 1136. [Medline] [CrossRef]
36. Simčič P, Lowe R, Granziera V, Pierini A, Torrigiani F, Lubas G. 2020. Electrochemotherapy in treatment of canine oral non-tonsillar squamous cell carcinoma. A case series report. *Vet Comp Oncol* **18**: 428–432. [Medline] [CrossRef]
37. Takeuchi S, Fukuda K, Yamada T, Arai S, Takagi S, Ishii G, Ochiai A, Iwakiri S, Itoi K, Uehara H, Nishihara H, Fujita N, Yano S. 2017. Podoplanin promotes progression of malignant pleural mesothelioma by regulating motility and focus formation. *Cancer Sci* **108**: 696–703. [Medline] [CrossRef]
38. Wolber P, Schwarz D, Niemczyk M, Drebber U, Klußmann JP, Meyer M. 2020. Expression of podoplanin correlates with prognosis in nasopharyngeal carcinoma. *Eur Arch Otorhinolaryngol* **277**: 1185–1190. [Medline] [CrossRef]
39. Wu Y, Liu Q, Yan X, Kato Y, Tanaka M, Inokuchi S, Yoshizawa T, Morohashi S, Kijima H. 2016. Podoplanin-mediated TGF-β-induced epithelial-mesenchymal transition and its correlation with bHLH transcription factor DEC in TE-11 cells. *Int J Oncol* **48**: 2310–2320. [Medline] [CrossRef]
40. Xia ZB, Meng FR, Fang YX, Wu X, Zhang CW, Liu Y, Liu D, Li GQ, Feng FB, Qiu HY. 2018. Inhibition of NF-κB signaling pathway induces apoptosis and suppresses proliferation and angiogenesis of human fibroblast-like synovial cells in rheumatoid arthritis. *Medicine (Baltimore)* **97**: e10920. [Medline] [CrossRef]
41. Yao J, Duan L, Fan M, Wu X. 2006. NF-kappaB signaling pathway is involved in growth inhibition, G2/M arrest and apoptosis induced by Trichostatin A in human tongue carcinoma cells. *Pharmacol Res* **54**: 406–413. [Medline] [CrossRef]