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NOTE

Clinical Pathology

Establishment of rat anti-canine DEP domain containing 1B (DEPDC1B) monoclonal antibodies

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ABSTRACT. DEP domain-containing 1B (DEPDC1B) is involved in the regulation of cell de-adhesion and actin cytoskeleton activity during the G2/M transition of the cell cycle, and its overexpression has been proven to be associated with cancer progression in several human cancers. Canine DEPDC1B was identified as a gene that was overexpressed in canine lymphoma tissues in our previous study. However, in dogs, the protein expression of DEPDC1B remains to be determined due to the lack of a specific monoclonal antibody. Here, we developed rat monoclonal antibodies against canine DEPDC1B and characterized their applicability for immunodetection assays. Our findings demonstrated that these antibodies are functional and can be important tools to investigate the precise role of DEPDC1B in canine tumors.

KEY WORDS: antibody, cancer, DEP domain-containing 1B, dog

The DEP domain-containing 1B (DEPDC1B) gene was recently identified and localized at human chromosome 5 (5q12.1). DEPDC1B contains two conserved domains, a DEP domain and Rho-GAP domain, which are involved in the Rho GTPase signaling pathway. Small GTPases of Rho family including Rho, Rac, and Cdc42 [13] regulate the assembly and disassembly of the actin cytoskeleton depending on the extracellular signaling [8]. Marchesi S. *et al.* showed that DEPDC1B accumulates in G2 phase of the cell cycle and controls entry into mitosis through the modulation of cell adhesion and actin cytoskeleton dynamics [6]. In addition, DEPDC1B has been reported to be overexpressed and associated with tumor cell migration, invasion, and tumor growth in various types of human cancers such as breast cancer [2], prostate cancer [1], non-small cell lung cancer [12], and malignant melanoma [11]. However, in dogs, the expression and the function of DEPDC1B remains largely unknown. Our recent analyses revealed that DEPDC1B mRNA is overexpressed in canine T cell lymphoma cells by cDNA microarray analysis (manuscript in preparation). Although DEPDC1B mRNA levels in canine lymphoma tissues were quantified by real-time PCR, evaluation of protein expression of DEPDC1B was not possible because of the lack of a specific monoclonal antibody. Even though we have verified the specificity of an anti-human DEPDC1B polyclonal antibody against canine DEPDC1B protein by the antigen pre-adsorption test, many non-specific bands were also observed. Therefore, in the present study, we established rat monoclonal antibodies that specifically detected the canine DEPDC1B protein. Then, we attempted to investigate the binding epitopes of these antibodies by western blotting and to identify their applicability for the immunodetection of DEPDC1B (i.e.: immunoprecipitation, immunohistochemistry, and immunocytochemistry).

First, to generate a recombinant glutathione-S-transferase (GST)-tagged protein of canine DEPDC1B in a bacterial system for immunization, we amplified a partial sequence of canine DEPDC1B (encoding amino acids (a. a.) 90–161) using specific primers 5′-GCCGAATTCGGAAAATGGGGTCAGGAAGA-3′ and 5′-GGCCTCGAGTTATACTGTGACGCTTATACCAC-3′ followed by incorporation into a pGEX-4T-1 vector (GE Healthcare Japan, Tokyo, Japan). Female Sprague-Dawley rats (Oriental Yeast, Tokyo, Japan) were immunized by injection of the recombinant GST-tagged partial canine DEPDC1B (encoding amino acids 90–161) into the hind foot pads and the tale head. One week after immunization, popliteal lymph nodes were removed and fused with a mouse

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Table 1. Screening and characterization of monoclonal antibodies against canine DEP domain-containing 1B (DEPDC1B) by ELISA, WB, IP, IHC and ICC

mAb Clone number	Canine DEPDC1B protein is detected by:						Epitope mapping
	ELISA	WB	IP	IHC in:		ICC	
				T lymphoma tissue	Normal tissue		
#1	+	+					108–125
#2	+	−					
#3	+	−					
#4	+	+	+	+	+ (Kidney) − (Lymph node)	+	90–125
#5	+	+	+	−			90–125
#6	+	+					90–125
#7	+	−					
#8	+	+					90–125
#9	+	+					90–125
#10	+	+					90–125
#11	+	+					90–125
#12	+	−					
#13	+	+	+	+			108–125
#14	+	+	+	−			90–125

ELISA, enzyme-linked immunosorbent assay; WB, western blotting; IP, immunoprecipitation; IHC, immunohistochemistry; ICC, immunocytochemistry. Blank columns indicate 'not tested'.

myeloma cell line P3X63Ag8 using polyethylene glycol (Roche, Basel, Switzerland). Hybridoma cells were cultured in RPMI1640 medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum (FBS) and hypoxanthine-aminopterin-thymidine (Thermo Fisher Scientific, Waltham, MA, USA) as described previously [9]. After colonies were formed, the supernatant from hybridomas was harvested and screened using an Enzymed-Linked ImmunoSorbent assay (ELISA) for positivity to the recombinant partial DEPDC1B protein, which was removed GST-tag using thrombin cleavage and ion exchange chromatography. Then, DEPDC1B-positive hybridomas were cloned three times using the limiting dilution method. Finally, the supernatant from each clone was collected and 14 monoclonal antibodies were purified using HiTrap Protein G HP columns (GE Healthcare UK, Buckinghamshire, England).

To confirm that these antibodies recognized the two-dimensional structure of DEPDC1B, western blotting analysis was performed using Madin-Darby canine kidney (MDCK) cells stably expressing canine DEPDC1B. To obtain this stable cell line, a lentiviral expression system was used, with a modification of a previously described technique [4]. We amplified the full length of DEPDC1B sequence from normal kidney cDNA using specific primers 5′-CGCCCAATCAGCGCCC-3′ and 5′-AATCACCCATTTGCTCAAGG-3′ followed by adding two FLAG tag sequences at the C-terminus. A lentiviral vector (pS-IV) cloned with full length canine DEPDC1B with pC-VSVG and p8.9QV was transfected into human embryonic kidney HEK293T cells. MDCK cells were infected with the lentivirus, and Venus fluorescent protein-positive MDCK cells were verified using an IX73 fluorescence microscope (OLYMPUS, Tokyo, Japan). Harvested cells were lysed with 1% NP40 lysis buffer {1% NP40, 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM EDTA} supplemented with a protease inhibitor cocktail (Nacalai Tesque, Kyoto, Japan). The resulting supernatants were collected and used as cell lysates in the following experiments. Each lysate was loaded on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and then western blotting was performed using our monoclonal antibodies. Mouse anti-beta actin monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) was used as a loading control. A horseradish peroxidase (HRP)-conjugated mouse anti-rat IgG polyclonal antibody (Zymed, Tokyo, Japan) was used as a secondary antibody, then the membrane was visualized using Western Lightning Chemiluminescence reagent (PerkinElmer, Waltham, MA, USA). As a result, we found that 10 out of 14 monoclonal antibodies specifically reacted with the DEPDC1B protein by western blotting (Table 1).

Next, we investigated the epitope mapping of monoclonal antibodies that reacted with DEPDC1B protein in the western blotting analysis, using recombinant GST-tagged DEPDC1B fragments. To generate three DEPDC1B fragments, pDEP-1 (a.a. 90–125), pDEP-2 (a.a. 125–161), and pDEP-3 (a.a. 108–143) (Fig. 1A) in the bacterial system, polymerase chain reaction amplification was performed using the primer pairs; YTM1694 (5′-ACGGATCCGGAAAATGGGGTCAGGAAGATTTTGAAG-3′) and YTM1697 (5′-GCCTCGAGAATACTGTGACGCTTATACCACATCTC-3′), YTM1695 (5′-ACGGATCCCCTTCTTCACCCCTGAAACC-3′) and YTM1698 (5′-GCCTCGAGGATGTTCTCCTGTGAAGTGCTCTG-3′), and YTM1696 (5′-ACGGATCCATTAAATTTCCCGAATGGAATGACCC-3′) and YTM1699 (5′-GCCTCGAGAACATCCTTTTGGTATGCAGGCTTC-3′), respectively. These products were inserted into a pGEX-4T-1 vector (GE Healthcare Japan) and used to transformed *E. coli* BL21 (DE3) pLysS (Promega, Madison, WI, USA) competent cells, followed by expression of three GST-tagged partial DEPDC1B proteins following the addition of 0.1 mM isopropyl beta-D-1-thiogalactopyranoside (IPTG). These bacteria were sonicated and purified proteins using glutathione sepharose 4B (GE Healthcare Japan). SDS-PAGE and western blotting were performed as described above. Each canine DEPDC1B monoclonal antibody was

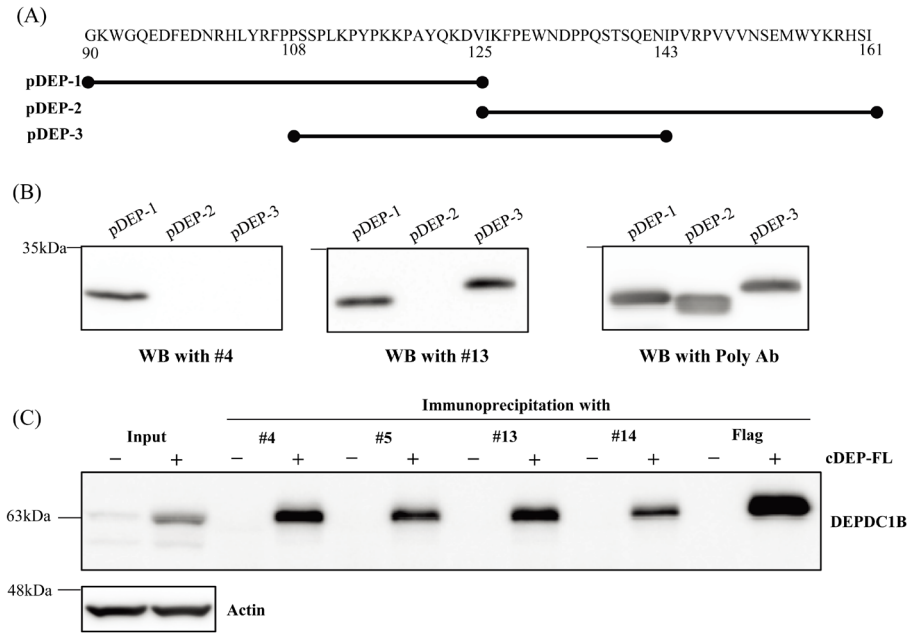

Fig. 1. Determination of the epitope of established monoclonal antibodies and immunoprecipitation analysis of DEP domain-containing 1B (DEPDC1B). (A) A schematic representation of recombinant partial DEPDC1B fragments fused with glutathione-S-transferase (GST) is shown. pDEP-1, pDEP-2, and pDEP-3 comprised amino acids 90–125, 125–161, and 108–143, respectively. (B) Western blotting analysis of recombinant partial DEPDC1B fragments (as shown in A) was performed using canine DEPDC1B monoclonal antibodies (#4 and #13) or anti-human DEPDC1B polyclonal antibody. (C) To assess the binding properties of anti-canine DEPDC1B monoclonal antibodies (#4, #5, #13, and #14) to FLAG-tagged canine DEPDC1B overexpressed in MDCK cells, an immunoprecipitation assay was performed. The cell lysates from MDCK cells expressing DEPDC1B or empty vector were immunoprecipitated with 1 μg of either anti-canine DEPDC1B monoclonal antibody or anti-FLAG M2 antibody. The input control protein (leftmost) and immunoprecipitated proteins (right) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane, followed by the western blotting analysis. To detect the band for DEPDC1B, we used an anti-human DEPDC1B polyclonal antibody, which was shown to cross-react against canine DEPDC1B. Actin was used as a loading control.

used as a primary antibody. Rabbit anti-human DEPDC1B polyclonal antibody (Sigma-Aldrich) was used as a positive control. Representative results of these western blotting analyses are shown in Fig. 1B. Monoclonal antibody #4 detected a band from the pDEP-1 fragment, but not pDEP-2 or pDEP-3. Monoclonal antibody #13 detected bands with both pDEP-1 and pDEP-3. This suggested that the sequence a.a. 108–125 was recognized by antibody #13. However, no antibodies detected pDEP-2. The data from the remaining clones are shown in Table 1.

In addition, to determine whether our established monoclonal antibodies bound directly with canine DEPDC1B, we performed immunoprecipitation assays. In the following experiments, we randomly picked up four clones (#4, #5, #13, and #14) among 10 clones which are available for the western blotting method. Each lysate from MDCK cells or MDCK cells overexpressing canine DEPDC1B was incubated with the selected canine DEPDC1B monoclonal antibodies or anti-FLAG M2 antibody (Sigma-Aldrich) accompanied with protein A/G agarose (Santa Cruz Biotechnology, Dallas, TX, USA) on a rotating wheel overnight at 4°C to immunoprecipitate the FLAG-tagged canine DEPDC1B protein. The samples were washed and loaded on a 10% SDS-PAGE gel, followed by the western blotting using the rabbit anti-human DEPDC1B polyclonal antibody (Sigma-Aldrich). HRP-conjugated donkey anti-rabbit IgG polyclonal antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used as a secondary antibody. We found that all four clones were able to immunoprecipitate canine DEPDC1B protein (Fig. 1C). These data suggest that our established monoclonal antibodies are applicable not only for western blotting analysis but also for immunoprecipitation analyses. Therefore, these antibodies might be useful in investigations of the function and protein–protein interaction of canine DEPDC1B in future studies.

To examine the binding properties of the selected monoclonal antibodies (#4, #5, #13, and #14) to canine DEPDC1B, which were able to recognize the three-dimensional structure of DEPDC1B by immunoprecipitation, immunohistochemistry and immunocytochemistry analyses were carried out using canine tissues. For the immunohistochemical staining, the formalin-fixed and paraffin-embedded canine T cell lymphoma tissue was treated with pH 6.0 citrate buffer for 30 sec at 125°C in a pressure cooker (Dako Japan, Tokyo, Japan) as antigen retrieval. Sections were incubated with each monoclonal antibody, followed by Histofine Simple Stain MAX-PO (Nichirei Biosciences, Tokyo, Japan) as described previously [7]. The sections were visualized using Peroxidase Stain DAB Kit (Nacalai Tesque) before counterstaining with Meyer's hematoxylin. Clones #5 and #14 did not cross-react with a canine T cell lymphoma tissue (Table 1). In contrast, the sections were positively stained with monoclonal

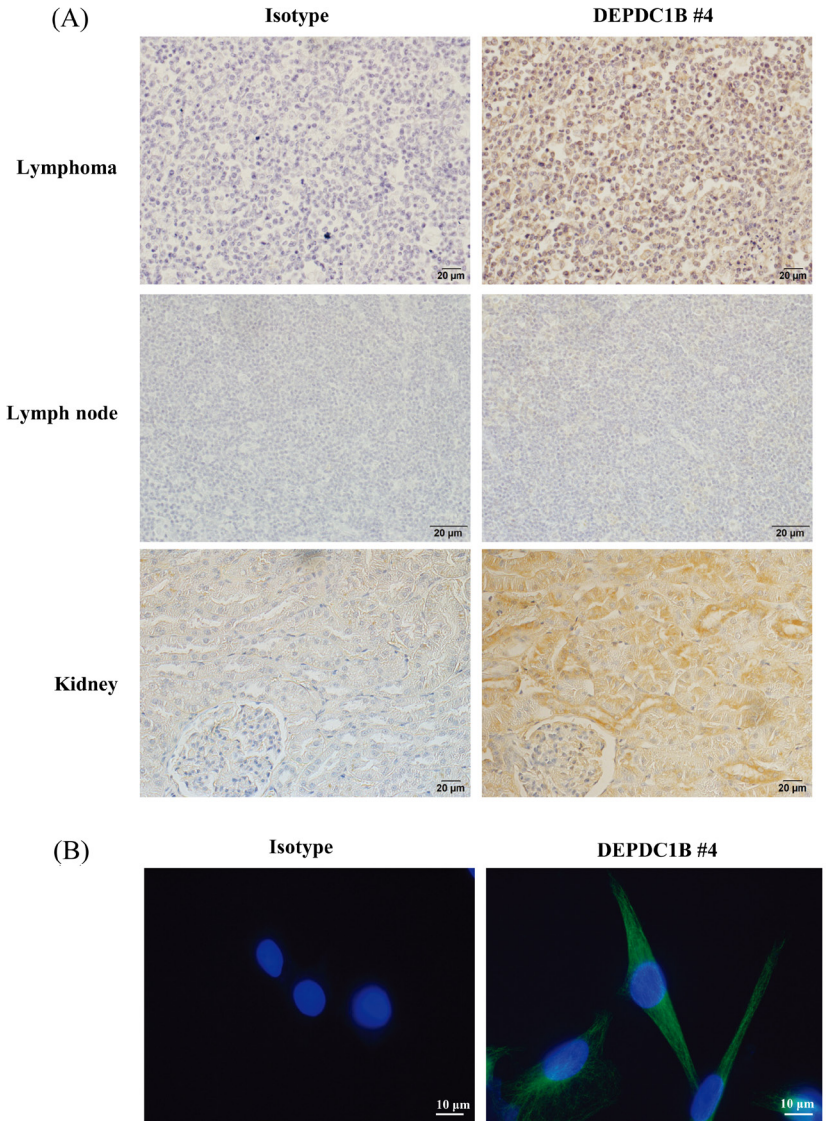

Fig. 2. Immunohistochemistry and immunocytochemistry of canine DEP domain-containing 1B (DEPDC1B). (A) DEPDC1B expression in a canine lymphoma, a normal lymph node, and a normal kidney was shown by immunohistochemical staining. Sections were incubated with anti-canine DEPDC1B monoclonal antibody clone #4 (right panel) or rat IgG2b isotype control (left panel). DEPDC1B protein was observed in the cytoplasm of lymphoma cells and renal tubules in a kidney tissue. Scale bar: 20 μm. (B) DEPDC1B expression in a canine mammary gland tumor cell line CHM-p was assessed by immunocytochemical staining. CHM-p was fixed, stained with anti-canine DEPDC1B monoclonal antibody clone #4 or rat IgG2b, followed by DyLight 488-labelled anti-IgG antibody. DEPDC1B protein was visualized using fluorescence microscopy. Nuclei were stained with DAPI. Scale bar: 10 μm.

antibody clones #4 and #13. DEPDC1B protein was located in the cytoplasm in the sections stained with clones #4 and #13, which was consistent with human studies [1, 11]. However, the section stained with clone #13 showed weaker positive staining compared with the section stained with clone #4 (data not shown). Referring to human studies for the detectability of DEPDC1B protein [1, 11], we selected clone #4 as an antibody suitable for immunohistochemical staining. Moreover, to assess the DEPDC1B expression in a canine T cell lymphoma tissue, a normal lymph node, and a normal kidney tissue, immunohistochemistry was performed using monoclonal antibody #4 (5 μg/m*l* final concentration) or normal rat IgG2b (Santa Cruz Biotechnology) as an isotype control. As shown in Fig. 2, canine DEPDC1B was highly expressed in a canine T cell lymphoma tissue, compared to a normal lymph node. These data, therefore, suggest that canine DEPDC1B may be involved in the tumorigenesis of canine T cell lymphoma. This contribution of DEPDC1B expression in canine lymphoma cells is currently under investigation. In addition, DEPDC1B was strongly stained and localized in the cytoplasm of renal tubules, which was consistent with the result of PCR amplification from kidney cDNA. For immunocytochemical analysis labeled with a fluorescent dye, a canine mammary gland tumor cell line CHM-p (kindly provided by Dr. Takayuki Nakagawa, The University of Tokyo) was cultured on microcover glasses (Matsunami Glass Kogyo, Osaka, Japan) in a 12-well tissue culture plate. On the following day, the cells were washed and fixed with 4% paraformaldehyde. The cells were then permeabilized by treatment with 0.1% Triton-X in phosphate-buffered saline (PBS), and treated with 5% bovine serum albumin in PBS to minimize a non-specific antibody binding. The sections were incubated with primary antibodies (anti-DEPDC1B monoclonal antibody #4 or normal rat IgG2b), and stained with DyLight 488-conjugated goat anti-rat IgG (Rockland Immunochemicals, Limerick, PA, USA). The coverslips were mounted on glass slides with Molecular Probes Prolong Gold with DAPI (Invitrogen, Carlsbad, CA, USA) for the staining of nuclei. Samples were visualized at 100× magnification using a BX53 fluorescence microscope (OLYMPUS, Tokyo, Japan). CHM-p was positively stained with anti-DEPDC1B monoclonal antibody #4 (Fig. 2B), which was consistent with the result in the formalin-fixed and paraffin-embedded canine mammary gland tumor tissues (data not shown). Even though we did not double-stain with actin

filaments, DEPDC1B protein might be located around the cell cytoskeleton due to the staining of linear structures in the cytoplasm. Therefore, these data suggest that canine DEPDC1B might have an essential role in mitosis in the cell cycle, which is similar to the function of human DEPDC1B [3, 6].

Although DEPDC1B protein has been analyzed extensively in human studies [1, 2, 5, 7, 10, 12], there are no reports about DEPDC1B expression in dogs, partly due to the lack of a specific monoclonal antibody against canine DEPDC1B. Some of the monoclonal antibodies established in the present study, particular clone #4, are promising for the analysis of canine DEPDC1B. The correlation between the protein expression levels of DEPDC1B and cancer progression has been described recently in human prostate cancer, non-small cell lung cancer, and malignant melanoma [1, 11, 12]. A tissue microarray of DEPDC1B in 73 prostate cancers demonstrated that high expression levels of DEPDC1B were significantly associated with advanced clinical stages [1]. DEPDC1B protein expression in non-small cell lung cancer and malignant melanoma tissues was found to be higher than that in normal lung tissues and normal skin tissues, respectively [11, 12]. Moreover, non-small cell lung cancer patients with DEPDC1B overexpression had poor outcomes (median survival time=27.8 months) compared with those with low DEPDC1B expressions (median survival time=39.1 months) [12]. In contrast, it was still unknown whether the expression of DEPDC1B in dogs is associated with cancer progression, to elucidate this we need to evaluate the expression levels of DEPDC1B in canine normal tissues as well as tumor tissue samples along with clinical information. The present study indicated that monoclonal antibody clone #4 was applicable for immunohistochemistry and immunocytochemistry. Hence, we hope that this antibody can contribute to further clinical investigations of cancer progression.

In conclusion, the present study established and validated 14 monoclonal antibodies against canine DEPDC1B. Furthermore, clone #4 was shown to be suitable for western blotting, immunohistochemistry, immunocytochemistry, and immunoprecipitation. However, the present study has two limitations. First, we could not reveal the expression profile of DEPDC1B in tumors as well as normal tissues in dogs, because the numbers of samples used in this study were not enough to conclude the expression profile of DEPDC1B. Thus, the further analysis of a large number of canine tumor and normal tissues is required. Second, the function of canine DEPDC1B, especially in canine tumor, is still unknown. Nevertheless, the newly established monoclonal antibody in this study demonstrated that canine lymphoma, canine mammary gland tumor, and normal kidney expressed canine DEPDC1B protein, and may help to identify the function and the mechanism of DEPDC1B protein in dogs. Therefore, this study provides an important tool for basic and clinical research in veterinary medicine.

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REFERENCES

1. Bai, S., Chen, T., Du, T., Chen, X., Lai, Y., Ma, X., Wu, W., Lin, C., Liu, L. and Huang, H. 2017. High levels of DEPDC1B predict shorter biochemical recurrence-free survival of patients with prostate cancer. *Oncol. Lett.* **14**: 6801–6808. [Medline]
2. Boudreau, H. E., Broustas, C. G., Gokhale, P. C., Kumar, D., Mewani, R. R., Rone, J. D., Haddad, B. R. and Kasid, U. 2007. Expression of BRCC3, a novel cell cycle regulated molecule, is associated with increased phospho-ERK and cell proliferation. *Int. J. Mol. Med.* **19**: 29–39. [Medline]
3. Chen, D., Ito, S., Hyodo, T., Asano-Inami, E., Yuan, H. and Senga, T. 2017. Phosphorylation of DEPDC1 at Ser110 is required to maintain centrosome organization during mitosis. *Exp. Cell Res.* **358**: 101–110. [Medline] [CrossRef]
4. Igase, M., Shibutani, S., Kurogouchi, Y., Fujiki, N., Hwang, C. C., Coffey, M., Noguchi, S., Nemoto, Y. and Mizuno, T. 2019. Combination therapy with reovirus and ATM inhibitor enhances cell death and virus replication in canine melanoma. *Mol. Ther. Oncolytics* **15**: 49–59. [Medline] [CrossRef]
5. Liu, P. Y., Tee, A. E., Milazzo, G., Hannan, K. M., Maag, J., Mondal, S., Atmadibrata, B., Bartonicek, N., Peng, H., Ho, N., Mayoh, C., Ciaccio, R., Sun, Y., Henderson, M. J., Gao, J., Everaert, C., Hulme, A. J., Wong, M., Lan, Q., Cheung, B. B., Shi, L., Wang, J. Y., Simon, T., Fischer, M., Zhang, X. D., Marshall, G. M., Norris, M. D., Haber, M., Vandesompele, J., Li, J., Mestdagh, P., Hannan, R. D., Dinger, M. E., Perini, G. and Liu, T. 2019. The long noncoding RNA lncNB1 promotes tumorigenesis by interacting with ribosomal protein RPL35. *Nat. Commun.* **10**: 5026. [Medline] [CrossRef]
6. Marchesi, S., Montani, F., Deflorian, G., D'Antuono, R., Cuomo, A., Bologna, S., Mazzoccoli, C., Bonaldi, T., Di Fiore, P. P. and Nicassio, F. 2014. DEPDC1B coordinates de-adhesion events and cell-cycle progression at mitosis. *Dev. Cell* **31**: 420–433. [Medline] [CrossRef]
7. Shosu, K., Sakurai, M., Inoue, K., Nakagawa, T., Sakai, H., Morimoto, M., Okuda, M., Noguchi, S. and Mizuno, T. 2016. Programmed cell death ligand 1 expression in canine cancer. *In Vivo* **30**: 195–204. [Medline]
8. Tapon, N. and Hall, A. 1997. Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. *Curr. Opin. Cell Biol.* **9**: 86–92. [Medline] [CrossRef]
9. Umeki, S., Suzuki, R., Shimojima, M., Ema, Y., Yanase, T., Iwata, H., Okuda, M. and Mizuno, T. 2011. Characterization of monoclonal antibodies against canine P-selectin glycoprotein ligand-1 (PSGL-1). *Vet. Immunol. Immunopathol.* **142**: 119–125. [Medline] [CrossRef]
10. Wu, D., Zhu, X., Jimenez-Cowell, K., Mold, A. J., Sollecito, C. C., Lombana, N., Jiao, M. and Wei, Q. 2015. Identification of the GTPase-activating protein DEP domain containing 1B (DEPDC1B) as a transcriptional target of Pitx2. *Exp. Cell Res.* **333**: 80–92. [Medline] [CrossRef]
11. Xu, Y., Sun, W., Zheng, B., Liu, X., Luo, Z., Kong, Y., Xu, M. and Chen, Y. 2019. DEPDC1B knockdown inhibits the development of malignant melanoma through suppressing cell proliferation and inducing cell apoptosis. *Exp. Cell Res.* **379**: 48–54. [Medline] [CrossRef]
12. Yang, Y., Liu, L., Cai, J., Wu, J., Guan, H., Zhu, X., Yuan, J. and Li, M. 2014. DEPDC1B enhances migration and invasion of non-small cell lung cancer cells via activating Wnt/β-catenin signaling. *Biochem. Biophys. Res. Commun.* **450**: 899–905. [Medline] [CrossRef]
13. Zohn, I. M., Campbell, S. L., Khosravi-Far, R., Rossman, K. L. and Der, C. J. 1998. Rho family proteins and Ras transformation: the RHOad less traveled gets congested. *Oncogene* **17**: 1415–1438. [Medline] [CrossRef]