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RESEARCH ARTICLE

Galectin-3 immunolabelling correlates with BCL2 expression in canine cutaneous mast cell tumours

THIAGO HENRIQUE M. VARGAS[1], CAMILA N. BARRA[1,2], LIDIA H. PULZ[1,2], GREICE C. HUETE[1], KARINE G. CADROBBI[1], ADRIANA TOMOKO NISHIYA[3], SILVIA REGINA KLEEB[3], JOSÉ GUILHERME XAVIER[4], JOSÉ LUIZ CATÃO-DIAS[2] and RICARDO F. STREFEZZI[1]*

[1] Laboratório de Oncologia Comparada e Translacional (LOCT), Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte 225, Jd. Elite, Pirassununga, CEP 13635-900, São Paulo, Brazil

[2] Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brazil

[3] Universidade Anhembi Morumbi, São Paulo, SP, Brazil

[4] Universidade Paulista, São Paulo, SP, Brazil

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ABSTRACT

Mast cell tumour (MCT) is the most frequent skin neoplasm in dogs. These tumours are characterised by variable behaviour and clinical presentation that make prognosis an important and challenging task in the veterinary practice. Galectin-3 (Gal-3) is known to influence several biological processes that are important in the cancer context and has been described as a prognostic marker for several human cancers. The aim of the present work was to characterise Gal-3 immunolabelling in canine cutaneous MCTs and to investigate its value as a prognostic marker for the disease. Thirty-four random cases of canine cutaneous MCT that were surgically treated with wide margins were included in this study. Gal-3 expression was evaluated using immunohistochemistry and the results were compared with the expression of apoptosis-related proteins, Ki67 index, histopathological grades, mortality due to the disease and post-surgical survival. The majority of the MCTs (65.8%) were positive for Gal-3. Gal-3 immunolabelling was variable among the samples (2.7%–86.8% of the neoplastic cells). The protein was located in the cytoplasm or in the cytoplasm and the nucleus. Gal-3 positivity was correlated with BCL2 expression ($P < 0.001$; $r = 0.604$), but not with Ki67 and BAX. No significant differences were detected between histological grades or in the survival analysis. Gal-3 expression correlates with BCL2 expression in MCTs. Although an efficient marker for several human neoplasms, the results presented herein suggest that Gal-3 immunolabelling is not an independent prognostic indicator for this disease.

KEYWORDS

apoptosis, dog, galectin-3, immunohistochemistry, mast cell tumour, prognosis

INTRODUCTION

Mast cell tumour (MCT) is the most frequent skin neoplasm in dogs, representing up to 21% of the cases (Cohen et al., 1974; Dobson et al., 2002; Welle et al., 2008; Kiupel, 2017). Older (8–9 years old) dogs and a variety of breeds such as Boxer, Boston Terrier, Labrador and Golden Retriever are considered predisposed (Dobson et al., 2002; London and Seguin, 2003; Kiupel, 2017). These tumours are characterised by variable behaviour and unreliable clinical

*Corresponding author. Tel.: +55-19-3565-6731.
E-mail: rstrefezzi@usp.br

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presentation that make prognosis an important and challenging task in the veterinary practice (Welle et al., 2008; Blackwood et al., 2012). The prognosis of this disease is currently estimated on the basis of histological grading, clinical staging, Ki67 index and KIT immunolabelling patterns (London and Seguin, 2003; Blackwood et al., 2012; Kandefer-Gola et al., 2015; Kiupel, 2017).

Galectin-3 (Gal-3) is a lectin that is known to influence several biological processes important in the cancer context such as adhesion, migration, invasion, angiogenesis, cell proliferation and apoptosis (Elad-Sfadia et al., 2004; Nakahara et al., 2005; Dumic et al., 2006; Funasaka et al., 2014). Gal-3 immunolabelling has been described as a prognostic marker for human thyroid, gastric, colorectal and prostate cancers (Cheng et al., 2004; Endo et al., 2005; Wang et al., 2009). This protein is also expressed by normal epithelial cells, leukocytes, neurons, fibroblasts and osteoclasts, and it is associated with different effects, depending on its cellular location. For example, cytoplasmic Gal-3 exerts an anti-apoptotic effect, by interacting with BCL2 and promoting mitochondrial membrane stabilisation. On the other hand, nuclear Gal-3 associates with Nucling protein, which leads to apoptosis (Davidson et al., 2002; Dumic et al., 2006).

Veterinary oncology studies have revealed that mammary carcinoma metastases in bitches show higher Gal-3 expression than the primary tumours, and higher serum levels of this protein were detected in such dogs (De Oliveira et al., 2010; Ribeiro et al., 2016). Our research group investigated the expression of Gal-3 in different canine tumour types and verified that its immunolabelling patterns are variable (Vargas et al., 2018). We also found that cytoplasmic Gal-3 expression correlates positively with post-surgical survival in dogs with oral melanomas, while nuclear expression showed a negative correlation with it (Vargas et al., 2019). Chen et al. (2006) reported that Gal-3 mediates mast cell degranulation, with consequent proinflammatory response by the JNK pathway (Chen et al., 2006). However, to the best of our knowledge, Gal-3 expression was not evaluated in canine cutaneous mast cell tumours as a potential prognostic marker.

The aim of the present work was to characterise Gal-3 expression in canine cutaneous mast cell tumours and to compare it with patient survival, histopathological grading, Ki67 index and expression of the apoptosis-related proteins BAX and BCL2.

MATERIALS AND METHODS

Thirty-eight formalin-fixed paraffin-embedded samples from 34 dogs were obtained from the Tumour Bank of the Laboratório de Oncologia Comparada e Translacional of the Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo (FZEA-USP, Pirassununga, SP, Brazil). Four-micrometre sections were obtained and stained with haematoxylin and eosin for confirmation of the diagnosis and histopathological grading according to Patnaik's and Kiupel's proposals (Patnaik et al., 1984; Kiupel et al., 2011). The inclusion criteria were: (1) wide surgical excision aiming for cure and no previous or adjuvant therapy; (2) availability of clinical data; (3) minimum follow-up of six months for censored cases. All information was obtained from medical records and interviews with owners and/or veterinarians. Live animals at the end of study, deaths unrelated to MCT, and interrupted clinical history were censored for statistical analysis. All procedures involving the use of animals were approved by the Animal Ethics Committee of FZEA-USP (#1005140116/2016) and owners provided informed client consent for the research.

Four-µm histological sections were placed onto silanised slides for immunohistochemistry. Epitope retrieval was achieved by heating the slides in citrate buffer (pH 6.0) for 20 min in a steamer (Gal-3 and BAX) or for 2 min in a pressure cooker (Ki67); and in TRIS EDTA buffer (pH 9.0) for 2 min in a pressure cooker (BCL2). Nonspecific antigen binding was blocked with 5% skimmed milk solution. Samples were incubated with anti-Gal-3 (mouse monoclonal, Abcam, clone A3A12, code ab2785, 1:1,000), anti-Ki67 (mouse monoclonal, clone MIB-1, Dako Cytomation Inc., 1:50), anti-BCL2 (mouse monoclonal, Abcam, code ab117115, 1:1,500) or anti-BAX (rabbit polyclonal, clone P-19, Santa Cruz Biotechnology, code sc-526, 1:200) antibodies in a moist chamber, overnight, at 4 °C. Secondary antibody (Easylink ONE®, Easypath, São Paulo, SP, Brazil) was applied at room temperature for 15 min. The reactions were revealed with 3,3'-diaminobenzidine (DAB) and counterstained with Harris' haematoxylin. The primary antibody was replaced with normal mouse or rabbit IgG, in the same dilution and under the same conditions as test samples for negative controls.

Gal-3 and Ki67 expressions were determined as the percentage of positive mast cells in five high-power fields (HPF) selected from areas with the highest percentage and/or intensity of immunolabelling ('hot spots') for each protein, avoiding areas of inflammation, necrosis and the borders of the histological section. Brown staining of the cytoplasm and/or nuclei was considered a positive reaction for Gal-3 evaluation. All positive and negative mast cells were counted in each image to determine the percentages. Gal-3 positivity was evaluated in both cytoplasmic and/or nuclear localisations. BCL2 and BAX expressions were evaluated semi-quantitatively according to Barra et al. (2018). All slides were evaluated using the image analysis software (ImageJ®, NIH, USA) and images were captured using a microscope connected to a high-definition digital camera (Leica DM500 and Leica ICC50-HD, Leica Microsystems, Heerbrugg, Switzerland).

Immunohistochemical results were compared with histological grades using the Mann–Whitney test or unpaired *t*-test. Correlation between variables was analysed using Pearson or Spearman correlation tests. Survival analysis was performed using the Kaplan-Meier method followed by the Mantel–Cox logrank test. The GraphPad Prism® software (version 8.2.1 for macOS, GraphPad Software, Inc.) was used and the significance level was regarded as 5%.

RESULTS

The majority of the study population was composed of male dogs (18/34, 52.9%) with a mean age of 9 ± 6.6 years (9.1 ± 3.0 for grade I, 8.9 ± 3.1 for grade II, and 10.0 ± 2.3 for grade III MCTs). Most of the dogs were mongrel dogs (9/34, 26.5%), followed by Boxer, Labrador (5/34 each, 14.7%) and Dachshund (4/34, 11.8%). The remaining dogs were Poodle, Shih Tzu, Pit Bull and Brazilian Fila (2/34, 5.9% each), Doberman Pinscher, Great Dane and Miniature Pinscher (1/34, 2.9% each). Mean post-surgical survival was 628 days (range 3 to 2,670 days). Twenty-five (73.5%) cases were censored (17 alive at the end of the study and eight deaths unrelated to MCT) and nine (26.5%) dogs died due to MCT. Ten MCTs were classified as grade I (26.3%), 19 as grade II (50%) and nine as grade III (23.7%) according to Patnaik et al. (1984); 27 (71.0%) were low-grade and 11 (29.0%) high-grade MCTs according to Kiupel et al. (2011).

Twenty-five (65.8%) MCTs were positive for Gal-3 and the protein was located in the cytoplasm or in the cytoplasm and the nucleus (Fig. 1). Positivity ranged from 2.7% to 86.8% of the neoplastic mast cells. According to Patnaik's grading system, 13.3 ± 21.2% (mean ± SD) of the neoplastic mast cells were positive in grade I MCTs, whereas 29.5 ± 30.9% and 28.7 ± 36.2% in grade II and III MCTs, respectively ($P = 0.364$). Using Kiupel's grading system for MCTs, 25.3 ± 29.2% of the cells were positive in low-grade and 24.5 ± 33.8% in high-grade tumours ($P = 0.948$). Moreover, no statistically significant differences were detected between dogs that died due to the disease (23.9 ± 31.3%) and censored cases (28.5 ± 31.3%; $P = 0.721$). A cut-off value of 1.93% was established using a ROC curve [sensitivity = 45.5%, specificity = 70.4%, area under the curve (AUC) = 0.529], but no significant differences were found in the survival analysis between the groups ($P = 0.731$, Fig. 2). A second ROC curve was plotted based on a two-year post-surgical survival (sensitivity = 25.0%, specificity = 92.3%, AUC = 0.613) and, similarly, no significant differences were found in the survival analysis between the groups ($P = 0.904$; cut-off = 60.23%).

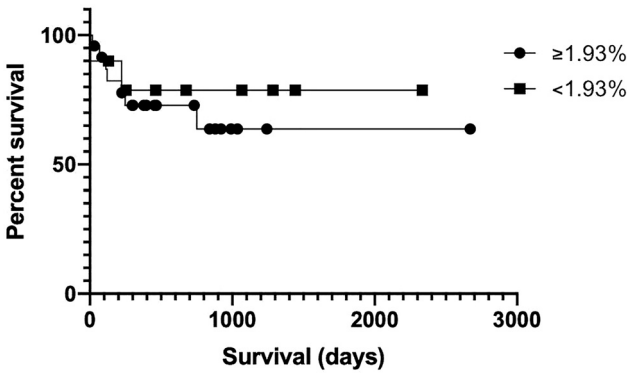

Fig. 2. Survival curves for mast cell tumours in dogs with Gal-3 positivity <1.93% and ≥1.93% ($P = 0.731$, chi-square = 0.292, median survival = undefined). Kaplan-Meier method, log-rank test

Thirty-three samples were available for BCL2 immunohistochemistry and 32 for BAX. These samples were used in a previous study of our research group which described the expression of apoptosis-related proteins in MCTs (Barra et al., 2018). The majority of the samples included here were positive for BCL2 (25/33, 75.8%), with low BCL2 expression in 54.5% (18/33) and high expression in 21.2% (7/33). All samples were positive for BAX, with 56.3% (18/32) showing high expression of this protein. Gal-3 immunolabelling was positively correlated with BCL2 ($P < 0.001$; $r = 0.604$), but not with BAX ($P = 0.790$) or Ki67 ($P = 0.333$). Although we found significant differences in Ki67 positivity between grades using both the grading system of Patnaik et al. (1984) and that of Kiupel et al. (2011) ($P = 0.011$ and $P = 0.013$, respectively), this marker was not an efficient indicator of survival when the cut-off of 1.8% (Scase et al., 2006) was used.

DISCUSSION

In the present study, we showed a positive correlation between Gal-3 and BCL2 immunolabelling, corroborating the

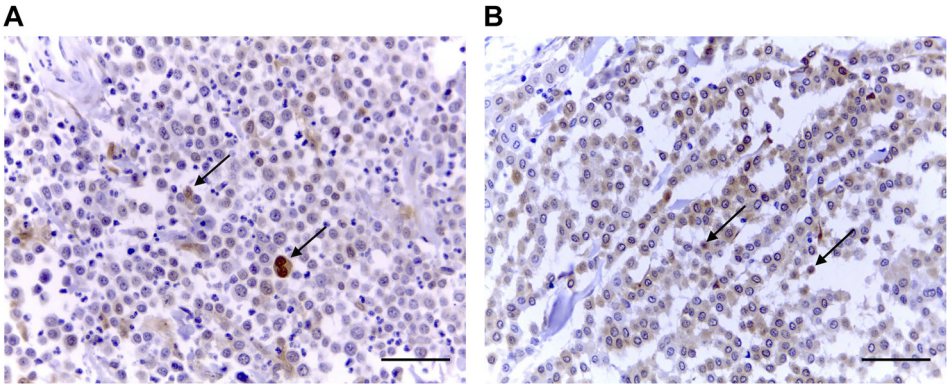

Fig. 1. Photomicrographs of canine mast cell tumours showing (a) low percentage of positive cells and low intensity for Gal-3 immunolabelling, with both cytoplasmic and nuclear labelling (arrows); and (b) high percentage of positive cells and strong staining intensity, with rare positive nuclei (arrows). Immunohistochemistry (IHC) for Gal-3. Bar = 50 µm

findings of other studies and the previous results of our research group on canine oral melanomas (Akahani et al., 1997; Liu et al., 2004; Harazono et al., 2014b; Vargas et al., 2019). Lower BCL2 expression has been detected in high-grade MCTs and it resulted in a higher risk of death due to the disease (Barra et al., 2018). Gal-3 contains an anti-death motif of the BCL2 family, forming a heterodimer that stabilises the mitochondrial membrane and avoids apoptosis (Akahani et al., 1997). Phosphorylated Gal-3 moves from the nucleus to the cytoplasm in order to protect breast cancer cells from apoptosis, which may contain an important mechanism of chemoresistance (Takenaka et al., 2004). Ser6 and Ser12 were described as the sites for Gal-3 phosphorylation, similarly to BCL2 (Mazurek et al., 2000). Synexin, a phospholipid-binding protein, is known to assist translocation to the mitochondria (Yu et al., 2002; Harazono et al., 2014b), where Gal-3 may influence the regulation of mitochondrial membrane stability by increasing the activity of certain protein kinases, such as pERK. This action prevents cytochrome C release and, thus, apoptosis (Takenaka et al., 2004).

Gal-3 immunolabelling was variable among samples and, although this protein has been demonstrated as a prognostic marker for several cancer types in humans (Cheng et al., 2004; Endo et al., 2005; Wang et al., 2009), as well as for some canine tumours (De Oliveira et al., 2010; Ribeiro et al., 2016; Vargas et al., 2019), it was not associated with survival, histopathological grading, BAX expression and Ki67 index in MCTs. Although we have investigated two cut-off points for Gal-3 expression, considering both minimum one- and two-year survival periods, the AUC for both tests was considered poor (between 0.6 and 0.7), as well as the survival curves were not significantly different between groups. These results show that the expression of Gal-3 in MCTs is not a good marker when analysed alone.

We also compared Gal-3 and BAX expressions, but no correlation was found between the immunolabelling of these proteins, in accordance with the findings of Huang et al. (2008). Gal-3 overexpression was negatively correlated with BAX in human pituitary tumours (Diao et al., 2018) and it was proposed that this lectin inhibits BAX oligomerisation, thus antagonising the apoptotic intrinsic pathway (Harazono et al., 2014a). Translocation from the nucleus to the cytoplasm also decreases BAD expression and increases BAD phosphorylation, resulting in the attenuation of mitochondrial membrane depolarisation (Inohara and Raz, 1995).

Since Gal-3 is frequently associated with cell proliferation (Elad-Sfadia et al., 2004; Nakahara et al., 2005; Dumic et al., 2006; Funasaka et al., 2014), we tested a possible correlation between Gal-3 immunolabelling and the Ki67 index, but no significant association was found. Similar results were reported by others for human gastric cancer, even though low Gal-3 expression was associated with poor prognosis in those patients, and by our research group for canine oral melanomas (Okada et al., 2006; Vargas et al., 2019). Cell cycle arrest is considered an important role of Gal-3 in cancer (Dumic et al., 2006). Downregulation of cyclins E and A, as well as upregulation of their inhibitors (p21 and p27), were reported in breast epithelial cancer cells that overexpressed Gal-3 (Kim et al., 1999). Others reported that the inhibition of Gal-3 expression was correlated with higher proliferation activity in prostate and colon cancer cell lines (Ellerhorst et al., 2002; Ose et al., 2012). On the other hand, an interaction between Gal-3 and one of the most important oncoproteins, K-Ras, was demonstrated as a cause for increased cell proliferation (Elad-Sfadia et al., 2004). Interestingly, although significant differences in Ki67 expression between histological grades were found in the present study, this proliferation marker did not efficiently indicate survival using the cut-off established by Scase et al. (2006) and recommended by the European Consensus on MCTs (Blackwood et al., 2012). These conflicting effects of Gal-3 in cell proliferation might be explained by different roles of this protein in the cell cycle, depending on cell type, and deserve more detailed investigation in future studies.

MCTs are important neoplasms in the veterinary practice due to their high prevalence, frequent aggressiveness and variability in survival rates. Even using consolidated prognostic markers, it is still challenging to estimate the behaviour of this tumour precisely. To the best of our knowledge, this is the first study to verify the value of Gal-3 immunolabelling in the prognosis of canine cutaneous MCTs. Conflicting results have been published on this protein in the literature, probably due to the multitude of influences it might exert on intracellular pathways. Possible Gal-3 mutations and/or interactions with BAX, BCL2 or even other proteins may alter their functions and modulate apoptosis in different ways. Another possibility deserving future investigation is that Gal-3 might have different effects depending on the stage of tumour progression, which was not evaluated in the present study. In conclusion, although Gal-3 is an efficient marker for several human neoplasms, the results presented herein suggest that Gal-3 immunolabelling is not a reliable independent indicator. More in-depth studies, with a larger number of samples, are needed to confirm our observations and also to clarify how this lectin influences other cancer features such as proliferation and evasion of apoptosis.

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REFERENCES

Akahani, S., Nangia-Makker, P., Inohara, H., Kim, H. R. and Raz, A. (1997): Galectin-3: a novel antiapoptotic molecule with

a functional BH1 (NWGR) domain of Bcl-2 family. Cancer Res. **57,** 5272–5276.

Barra, C. N., Macedo, B. M., Cadrobbi, K. G., Pulz, L. H., Huete, G. C., Kleeb, S. R., Xavier J. G., Catão-Dias, J. L., Nishiya, A. T., Fukumasu, H. and Strefezzi, R. F. (2018): Apoptotic intrinsic pathway proteins predict survival in canine cutaneous mast cell tumours. Vet. Comp. Oncol. **16,** E38–E44.

Blackwood, L., Murphy, S., Buracco, P., De Vos, J. P., De Fornel-Thibaud, P., Hirschberger, J., Kessler, M., Pastor, J., Ponce, F., Savary-Bataille, K. and Argyle, D. J. (2012): European consensus document on mast cell tumours in dogs and cats. Vet. Comp. Oncol. **10,** e1–e29.

Chen, H-Y., Sharma, B. B., Yu, L., Zuberi, R., Weng., I-C., Kawakami, Y., Kawakami, T., Hsu, D. K. and Liu, F-T. (2006): Role of galectin-3 in mast cell functions: Galectin-3-deficient mast cells exhibit impaired mediator release and defective JNK expression. J. Immunol. **177,** 4991–4997.

Cheng, C. S., Dong, W. G., Luo, H. S. and Yu, B. P. (2004): Expression of galectin-3 and its significance in gastric carcinoma. Chin. J. Cancer Res. **16,** 176–181.

Cohen, D., Reif, J. S., Brodey, R. S. and Keiser, H. (1974): Epidemiological analysis of the most prevalent sites and types of canine neoplasia observed in a veterinary hospital. Cancer Res. **34,** 2859–2868.

Davidson, P. J., Davis, M. J., Patterson, R. J., Ripoche, M-A., Poirier, F. and Wang, J. L. (2002): Shuttling of galectin-3 between the nucleus and cytoplasm. Glycobiology **12,** 329–337.

De Oliveira, J. T., De Matos, A. I. F., Gomes, J., Vilanova, M., Hespanhol, V., Manninen, A., Rutteman, A., Chammas, R., Gärtner, F. and Bernardes, E. S. (2010): Coordinated expression of galectin-3 and galectin-3-binding sites in malignant mammary tumors: Implications for tumor metastasis. Glycobiology **20,** 1341–1352.

Diao, B., Liu, Y., Xu, G. Z., Zhang, Y., Xie, J. and Gong, J. (2018): The role of galectin-3 in the tumorigenesis and progression of pituitary tumors. Oncol. Lett. **15,** 4919–4925.

Dobson, J. M., Samuel, S., Milstein, H., Rogers, K. and Wood, J. L. N. (2002): Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. J. Small Anim. Pract. **43,** 240–246.

Dumic, J., Dabelic, S. and Flögel, M. (2006): Galectin-3: An open-ended story. Biochim. Biophys. Acta **1760,** 616–635.

Elad-Sfadia, G., Haklai,. R, Balan, E., Kloog, Y. (2004): Galectin-3 augments K-ras activation and triggers a ras signal that attenuates ERK but not phosphoinositide 3-kinase activity. J. Biol. Chem. **279,** 34922–34930.

Ellerhorst, J. A., Stephens, L. C., Nguyen, T. and Xu, X-C. (2002): Effects of galectin-3 expression on growth and tumorigenicity of the prostate cancer cell line LNCaP. Prostate **50,** 64–70.

Endo, K., Kohnoe, S., Tsujita, E., Watanabe, A., Nakashima, H., Baba, H. and Maehara, Y. (2005): Galectin-3 expression is a potent prognostic marker in colorectal cancer. Anticancer Res. **25,** 3117–3121.

Funasaka, T., Raz, A. and Nangia-Makker, P. (2014): Galectin-3 in angiogenesis and metastasis. Glycobiology **24,** 886–891.

Harazono, Y., Kho, D. H., Balan, V., Nakajima, K., Zhang, T., Hogan, V. and Raz, A. (2014a): Galectin-3 leads to attenuation of apoptosis through Bax heterodimerization in human thyroid carcinoma cells. Oncotarget **5,** 9992–10001.

Harazono, Y., Nakajima, K. and Raz, A. (2014b): Why anti-Bcl-2 clinical trials fail: a solution. Cancer Metastasis Rev. **33,** 285–294.

Huang, C-X., Hou, Y-H. and Liu, Y-S. (2008): Expression of galectin-3 correlates with apoptosis in pituitary adenoma cells. Neurosci. Bull. **24,** 34–38.

Inohara, H. and Raz, A. (1995): Functional evidence that cell surface galectin-3 mediates homotypic cell adhesion. Cancer Res. **55,** 3267–3271.

Kandefer-Gola, M., Nowak, M., Madej, J. A., Dzimira, S., Ciaputa, R. and Janus, I. (2015): Useful immunohistochemical indicators in canine mast cell tumours. Acta Vet. Hung. **63,** 49–59.

Kim, H. R., Lin, H. M., Biliran, H. and Raz, A. (1999): Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells. Cancer Res. **59,** 4148–4154.

Kiupel, M. (2017): Mast cell tumors. In: Meuten, D. J. (ed.) Tumors in Domestic Animals. John Wiley & Sons, Inc., Ames, USA. pp. 176–202.

Kiupel, M., Webster, J. D., Bailey, K. L., Best, S., DeLay, J., Detrisac, C. J., Fitzgerald, S. D., Gamble, D., Ginn, P. E., Goldschmidt, M. H., Hendrick, M. J., Howerth, E. W., Janovitz, E. B., Langohr, I., Lenz, S. D., Lipscomb, T. P., Miller, M. A., Misdorp, W., Moroff, S., Mullaney, T. P., Neyens, I., O'Toole, D., Ramos-Vara, J., Scase, T. J., Schulman, F. Y., Sledge, D., Smedley, R. C., Smith, K., Snyder, P., Southorn, E., Stedman, N. L., Steficek, B. A., Stromberg, P. C., Valli, V. E., Weisbrode, S. E., Yager, J., Heller, J. and Miller, R. (2011): Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet. Pathol. **48,** 147–155.

Liu, L., Sakai, T., Sano, N. and Fukui, K. (2004): Nucling mediates apoptosis by inhibiting expression of galectin-3 through interference with nuclear factor kappaB signalling. Biochem. J. **380,** 31–41.

London, C. A. and Seguin, B. (2003): Mast cell tumors in the dog. Vet. Clin. North Am. Small Anim. Pract. **33,** 473–489.

Mazurek, N., Conklin, J., Byrd, J. C., Raz, A. and Bresalier, R. S. (2000): Phosphorylation of the beta-galactoside-binding protein galectin-3 modulates binding to its ligands. J. Biol. Chem. **275,** 36311–36315.

Nakahara, S., Oka, N. and Raz, A. (2005): On the role of galectin-3 in cancer apoptosis. Apoptosis **10,** 267–275.

Okada, K., Shimura, T., Suehiro, T., Mochiki, E. and Kuwano, H. (2006): Reduced galectin-3 expression is an indicator of unfavorable prognosis in gastric cancer. Anticancer Res. **26,** 1369–1376.

Ose, R., Oharaa, O. and Nagase, T. (2012): Galectin-1 and galectin-3 mediate protocadherin-24-dependent membrane localization of β-catenin in colon cancer cell line HCT116. Curr. Chem. Genomics **6,** 18–26.

Patnaik, A. K., Ehler, W. J. and MacEwen, E. G. (1984): Canine cutaneous mast cell tumor: Morphologic grading and survival time in 83 dogs. Vet. Pathol. **21,** 469–474.

Ribeiro, C., Santos, M. S., De Matos, A. J., Barros, R., Gärtner, F., Rutteman, G. R. and De Oliveira, J. T. (2016): Serum galectin-3 levels in dogs with metastatic and nonmetastatic mammary tumors. In Vivo **30,** 13–16.

Scase, T. J., Edwards, D., Miller, J., Henley, W., Smith, K., Blunden, A. and Murphy, S. (2006): Canine mast cell tumors: Correlation of apoptosis and proliferation markers with prognosis. J. Vet. Intern. Med. **20,** 151–158.

Takenaka, Y., Fukumori, T., Yoshii, T., Oka, N., Inohara, H., Kim, H. R., Bresalier, R. S. and Raz, A. (2004): Nuclear export of phosphorylated galectin-3 regulates its antiapoptotic activity in response to chemotherapeutic drugs. Mol. Cell Biol. **24,** 4395–4406.

Vargas, T. H. M., Pulz, L. H., Ferro, D. G., Sobral, R. A., Venturini, M. A. F. A., Corrêa, H. L. and Strefezzi, R. F. (2019): Galectin-3 expression correlates with post-surgical survival in canine oral melanomas. J. Comp. Pathol. **173,** 49–57.

Vargas, T. H. M., Pulz, L. H. and Strefezzi, R. F. (2018): Immunohistochemical expression of galectin-3 in canine tumors. Braz. J. Vet. Pathol. **11,** 1–6.

Wang, Y., Naggia-Makker, P., Tait, L., Balan, V., Hogan, V., Pienta, K. J. and Raz, A. (2009): Regulation of prostate cancer progression by galectin-3. Am. J. Pathol. **174,** 1515–1523.

Welle, M. M., Bley, C. R., Howard, J. and Rufenacht, S. (2008): Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. Vet. Dermatol. **19,** 321–339.

Yu, F., Finley, R. L., Raz, A. and Kim, H-R. C. (2002): Galectin-3 translocates to the perinuclear membranes and inhibits cytochrome c release from the mitochondria – A role for synexin in galectin-3 translocation. J. Biol. Chem. **277,** 15819–15827.