NOTE *Pathology*

Immunohistochemical Detection of Growth Hormone (GH) in Canine Hepatoid Gland Tumors

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ABSTRACT. The aim of this study was to detect immunohistochemically means growth hormone (GH) in 24 hepatoid gland adenomas and 5 hepatoid gland carcinomas and to compare the difference of immunoreactivity between types of tumors. The tumors were classified according to the WHO standards. Tissue sections which were prepared from formalin-fixed, paraffin wax-embedded tissues from 25 male and 4 female dogs were carried out immunostaining using polyclonal primary anti-hGH and EnVision method. Of 24 hepatoid gland adenomas (perianal gland adenomas) 23 (95.8%) were positive. All 5 hepatoid gland carcinomas (perianal gland carcinomas) were positive. No statistically significant differences in percentage of labelled cells between malignant and benign tumors were seen. The present demonstration of GH in hepatoid gland tumors adds new data on GH in extra-pituitary tissues and hormon-dependent tumors.
KEY WORDS: canine, growth hormone, perianal gland tumor.

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Hepatoid gland adenoma (perianal gland adenoma) is a very common benign tumor of the modified sebaceous glands comprising more than 80% of hepatoid gland tumors and represents the third most common tumor in intact male dogs [19]. In contrast, hepatoid gland carcinomas (perianal gland carcinomas) are much less frequent than benign counterpart [19]. On the basis of the presence of androgen and estrogen hormone receptors [3, 17] and of a good post-surgical response to orchiectomy [18], it is generally accepted that hepatoid gland tumors represent an hormone-dependent system. Growth hormone (GH) expression has been reported in extrapituitary sites during normal and pathological conditions in other hormone-dependent settings such as hyperplastic and neoplastic mammary tissues of bitches [13, 16], in normal canine prostate [5] and in cystic endometrial hyperplasia [8]. Tissue proliferation can be induced by endogenous and exogenous GH [10] in canine mammary gland. Furthermore, ovariectomy at an early age exerts a major protective effect [15]. It is therefore possible that GH may be involved in neoplastic proliferation in other hormone-dependent tissues by providing a highly proliferative environment. The aim of the present study was to verify the immunodetection of GH in hepatoid gland adenomas (perianal gland adenomas) and hepatoid gland carcinomas (perianal gland carcinomas).

Twenty-nine samples from canine hepatoid gland tumors were obtained from private animal hospitals. No cases had received chemotherapy, radiation or other previous surgery before biopsy. Paraffin sections fixed in 10% neutral buffered formalin were stained with hematoxylin and eosin. Tumor types were classified according to the histopathologic criteria previously defined by World Health Organization [6]. Twenty-four out of 29 (82.76%) samples were classified as hepatoid gland adenomas (perianal gland adenomas) , and 5/29 (17.24%) were found to be hepatoid gland carcinomas (perianal gland carcinomas). No case of perianal gland epithelioma was included. For immunohistochemistry, paraffin sections 3 μm in thickness were mounted on poly-L-lysine slide (O. Kindler, GMBH, Ziegelhofstabe, Freiburg, Germany). Rabbit polyclonal antibody against GH (1:300, A570, DAKO Corporation, Carpinteria, CA, U.S.A.) reacting to human GH was used as primary antibody. As indicated by the producer the antibody reactivity by radioimmunoassay, was calculated to be 100% for hGH, 1% for prolactin, and less than 1% for hPL, FSH, TSH and LH. In the specification sheet it is indicated a cross reactivity of the antibody with GH in baboon, dog, gerbil, guinea pig, hamster, monkey and marmoset. Endogenous peroxidase activity was quenched by immersion of the slides in 3% hydrogen peroxide in methanol for 30 min at room temperature. Immunostaining was performed by using the EnVision+TM peroxidase anti-rabbit (DAKO) with hydrated in 10% sodium citrate buffer (pH 6.0) microwave oven pre-treatment. Tissue sections were incubated at room temperature for 60 min with primary antibody and then with the EnVision polymer for 30 min at room temperature. Sections were stained for 10 min with 3,3'-diaminobenzidine tetrahyrochloride (DAB SK-4100, Vector Laboratories, Inc., Burlingame, CA, U.S.A.), and counterstained with hematoxylin. For positive controls, 10% neutral buffered formalin-fixed paraffin sections of normal pituitary gland were used for GH. Ten % neutral buffered formalin-fixed paraffin sections of canine spleen were included with each run of antibody development and resulted in no staining. Skin biopsies which were sampled from perianal region including hepatoid glands and processed to yield 10% neutral buffered formalin-fixed paraffin sections, were included with each run of antibody development and resulted negative (Fig. 1). Negative controls sections treated with no primary antibody anti-hGH were included at each run of antibody development, and resulted in no detectable staining. The percentage of positively

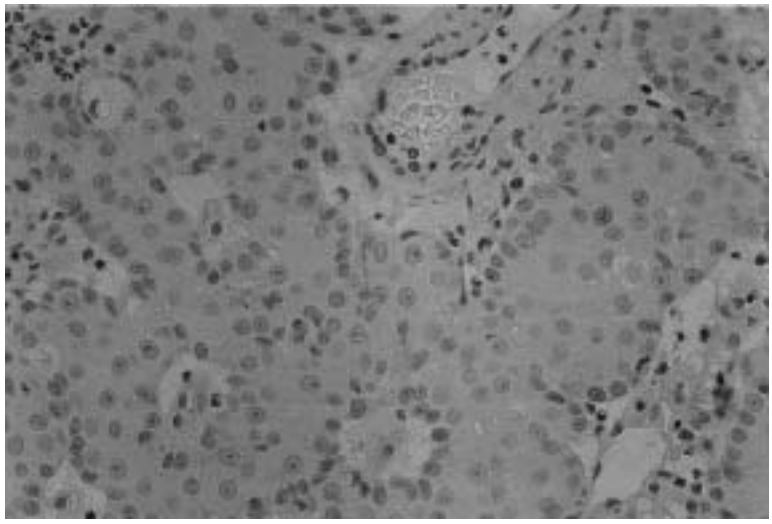

Fig. 1. Normal anti-GH negative canine hepatoid glands (original magnification, × 400).

Table 1. Immunoreactivity (anti-GH) in canine hepatoid gland tumors

	No. of cases	% of positive cells			
		Mean ± SD*	Median	Min	Max
Hepatoid gland adenomas	24	51.00 ± 29.45	49.25	0	99.50
Hepatoid gland carcinomas	5	53.60 ± 29.32	66.30	12,20	84,50

* SD = Standard Deviation.

stained tumor cells were evaluated by examining 10 representative areas at a magnification of 400. The ratio of number of positive tumor cells to negative cells was counted for each tissue area. A descriptive statistical analysis [1] was performed evaluating mean, standard deviation, median, minimum value and maximum value for each sample. Normality of data was assessed by the Kolmogorov-Smirnov test. Differences in percentage of labelled cells between hepatoid gland carcinomas (perianal gland carcinomas) and hepatoid gland adenomas (perianal gland adenomas) were evaluated by analysis of variance after arc-sin transformation of data, including age and sex as covariants. We also evaluated if age and percentage of positive cells may be predictive variables for type of neoplasia using the logistic multivariable regression. For all statistical analyses, a P value <0.05 was considered to be significant.

In the present study the occurrence of hepatoid gland carcinoma (perianal gland carcinoma) was higher than reported in literatue and may be explained with the low number of cases examined. The immunohistochemical results are summarized in Table 1. The sections from the canine pituitary gland were used to check anti-GH antibody cross-react. There was positive, granular, cytoplasmic staining in approximately 75% of adenohypophysis cells (Fig. 2). Twenty-eight (96.6%) of 29 hepatoid tumors were positive to GH. Of 24 hepatoid gland adenomas (perianal gland adenomas), 23 (95.8%) were positive. All 5 hepatoid gland carcinomas (perianal gland carcinomas) were positive. No significant differences were found by analysis of variance in percentage of positive tumor cells to anti-GH antibody between hepatoid gland adenomas (perianal gland adenomas) and hepatoid gland carcinomas (perianal gland carcinomas) (P=0.23). No significant association was found in the percentage of GH-positive cells between age (P=0.48) and tumor type (P=0.88). In all tissue examined anti-GH immunoreactivity was found to be granular and intense in the cytoplasm as that observed in adenohypophysis cells.

In this study, we first reported that GH was present in hepatoid gland adenomas (perianal gland adenomas) and hepatoid gland carcinomas (perianal gland carcinomas) in the dog and these tumors were GH tumors hormone-dependent. It was reported [10–12] that neoplastic cells in canine mammary gland tumors could produce GH at under continuous stimulation of progesterone or after malignant transformation. It was suggested that malignant cells became to bear a GH-producing potential without continuous progesterone stimulation.

It was reported that hepatoid glands possessed sex hormone receptors [3, 17] suspecting to be hormone dependent was suspected. In support of the observation, castration has long been a common practice to control hepatoid gland tumors [18]. We suspected that GH might be a mediator and/or may contribute to tumor growth and/or evolution. The role of GH in tumor biology was suspected in some

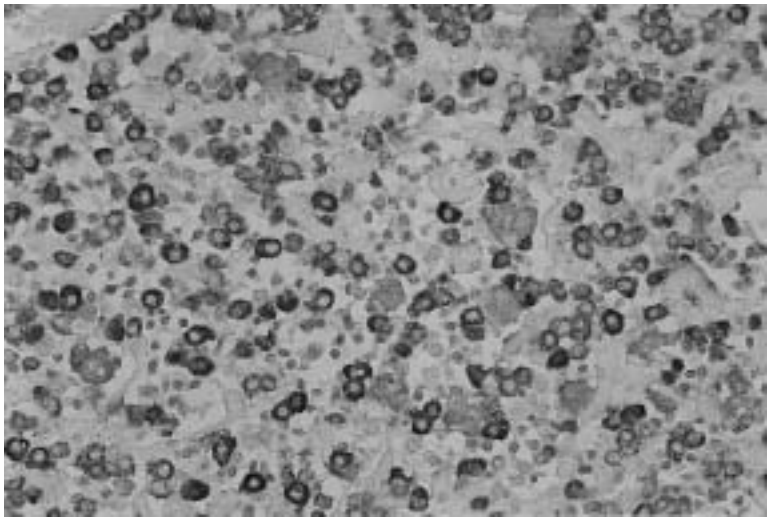

Fig. 2. Canine adenohyphophysis: note the high number of labelled cells with granular and intense anti-GH reactivity (original magnification, × 400).

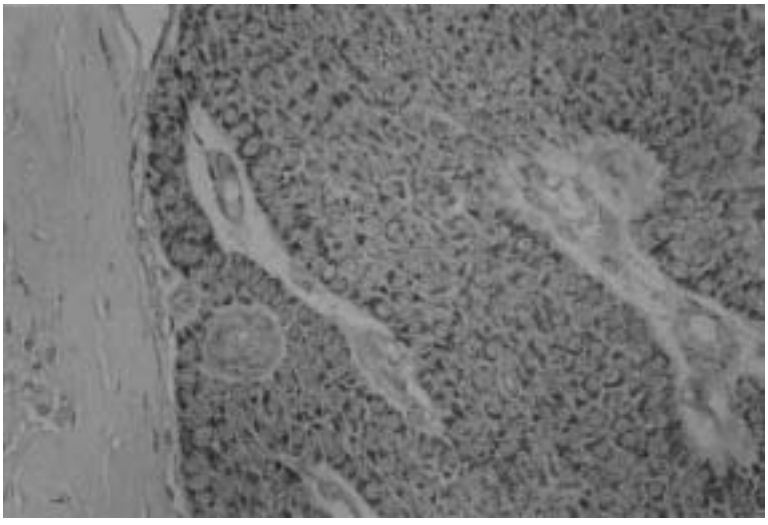

Fig. 3. Canine hepatoid gland adenoma: moderate anti-GH staining in cytoplasm of a cluster cells (original magnification, × 200).

human and animal tumors and in cell cultures. GH has been immunodetected in human cancers of the lung and stomach [2] and in laryngeal squamous cell carcinoma [4]. GH was associated with areas of hyperplastic mammary epithelium and of canine mammary gland tumors [16]. GH gene expression has been reported also in lymph nodes and lymphoma (in ref. 9 cited), although GH secretion itself was low [9]. The potential role of GH in carcinogenesis is also supported describing that carcinogenicity by dimethylbenz[a]anthracene [DMBA] was alleviated in transgenic mice expressing a growth hormone antagonist [14]. Kaulsay and colleagues [7] have demonstrated autocrine production of human growth hormone (hGH) in the MCF-7 mammary carcinoma cell line which was transfected with a plasmid encoding the hGH gene *in vitro*.

This immunohistochemical study may help in studying the pathogenesis of hormone dependent proliferative lesions and in exploring the topography of tissue localization ia a hormone. This study is preliminary and further investigations are needed to understand the biological role of GH in tumors, the GH production in the tissue, and the relationship between receptor expression and hormonal status (endocrine or secondary to pharmacological treatment) to clarify the pattern of expression and to evaluate its possible prognostic value and therapeutic implications.

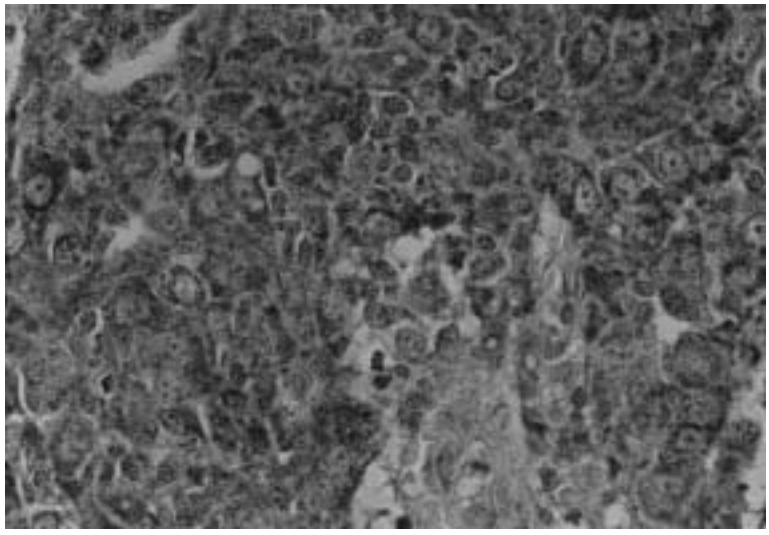

Fig. 4. Canine hepatoid gland carcinoma: intense cytoplasmic anti-GH immunoreactivity (original magnification, × 400).

REFERENCES

1. Armitage, P., Berry, G. and Matthews, J.N.S. 2001. Statistical Methods in Medical Research. Blackwell Science, Oxford.
2. Beck, C. and Burger, H.G. 1972. *Cancer* **30**: 75–79.
3. Chaisiri, N. and Pierrepoint, C.G. 1979. *J. Small Anim. Pract.* **20**: 405–416.
4. Deineko, G.M. and Kvetnoi, I.M. 1983. *Arkh. Patol.* **45**: 41–45.
5. El Etreby, M.F. and Mahrous, A.T. 1979. *Histochemistry* **67**: 279–286.
6. Goldschmidt, M.H., R.W. Dunstan, R.W., Stannard, A.A., von Tscharner, C., Walder, E.J. and Yager, J.A. 1998. Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals. Second series, Volume III, eds., Armed Forces Institute of Pathology, Washington.
7. Kaulsay, K.K., Mertani, H.C., Tornell, J., Morel, G., Lee, K.O. and Lobie, P.E. 1999. *Exp. Cell Res.* **250**: 35–50.
8. Kooistra, A.C., Mol, J.A., van Garderen, E., Kirpensteijn, J. and Rijnberk, A. 1997. *J. Reprod. Fertil.* **51**: 355–361.
9. Lantinga-van Leeuwen, I.S., van Garderen, E., Rutteman, G.R. and Mol, J.A. 2000. *J. Steroid Biochem. Molec. Biol.* **75**: 219–228.
10. Mol, J.A., van Garderen, E., Rutteman, G.R., and Rijnberk, A. 1996. *J. Steroid Biochem. Molec. Biol.* **57**: 67–71.
11. Mol, J.A., Selman, P.J., Sprang, E.P., van Neck, J.W. and Oosterlaken-Dijkstehuis, M.A. 1997. *J. Reprod. Fertil.* **51**: 339–344.
12. Mol, J.A., Lantinga van Leeuwen, I.S., van Garderen, E., Selman, P., Oosterlaken-duksterhuis, M.A., Schalken, J.A. and Rijnberk, A. 1999. *Vet. Q.* **21**: 111–115.
13. Petterino, C., Bovo, R., Zappulli, V., Martini, M., Poppi, L. and Castagnaro, M. 2001. GH expression in canine mammary gland tumors and hyperplasia. European Society of Veterinary Pathology, 19th Thessaloniki [Meeting abstract].
14. Pollak, M., Blouin, M.J., Zhang, J.C. and Kopchick, J.J. 2001. *Br. J. Cancer* **85**: 428–430.
15. Schneider, R., Dorn, C.R. and Taylor, D.O.N. 1969. *J. Natl. Cancer Inst.* **43**: 1249–1261.
16. van Garderen, E., de Wit, M., Voorhout, W.F., Rutteman, G.R., Mol, J.A., Nederbragt, H. and Misdorp, W. 1997. *Am J. Pathol.* **150**: 1037–1047.
17. Vercelli, A. 1997. Immnunohistochemical identification of estrogen and androgen receptors in canine hepatoid adenoma (PAA). Proceedings of 14th Annual Congress ESVD-ECVD, Pisa [Meeting abstract].
18. Wilson, G.P. and Hayes, H.M. 1979. *JAVMA* **174**: 1301–1303.
19. Withrow, S.J. and MacEwen, E.G. 1996. pp 261–267. *In*: Small Animal Clinical Oncology, 2nd. ed., Philadelphia, W.B. Saunders Company.