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.

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 literature and may be explained with the low number of cases examined. 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 literature 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 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] suggesting 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

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

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>% of positive cells Mean ± SD*</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoid gland adenomas</td>
<td>24</td>
<td>51.00 ± 29.45</td>
<td>49.25</td>
<td>0</td>
</tr>
<tr>
<td>Hepatoid gland carcinomas</td>
<td>5</td>
<td>53.60 ± 29.32</td>
<td>66.30</td>
<td>12.20</td>
</tr>
</tbody>
</table>

* SD = Standard Deviation.
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 in 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.
REFERENCES


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