VEGF-A and VEGFR-2 expression in canine cutaneous histiocytoma

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INTRODUCTION

Canine histiocytic proliferative disorders include lesions such as canine cutaneous histiocytoma (CCH), a common and usually solitary, benign neoplasm that appears as a rapidly growing, alopecic, erythematous, dome-shaped nodule, often with ulceration. The regression phenomena, to which is associated, makes it an attractive system for analysis of Langerhans cell histiocytosis behaviour, and could be regarded as an unique model to understand the pathogeny of the enigmatic disease of human Langerhans cell histiocytosis. VEGF (vascular endothelial growth factor), the most potent and ubiquitous endothelial growth factor constitutes a key sign used by oxygen private cells to promote growth and differentiation of blood vessels. It has a preponderant role in development of neoplasia and could determine tumour progression or regression. The aim of our study was to investigate if a lack of an appropriated vascular supply, due to an insufficient production of VEGF, could be associated to CCH spontaneous regression.

MATERIAL AND METHODS

HISTOPATHOLOGY AND TUMOURS CLASSIFICATION

Fifty tumours obtained from UTAD Histopathology Laboratory archives were analyzed in this study. CCH were classified into 4 histological groups according to lymphoid infiltrate, representing different stages of regression; 18 CCHs were classified as groups I, 13 as group II, 12 as group III and 7 as group IV. In group I lymphocytic infiltrate was minimal and peripheral; in group II, lymphocytic infiltrate was moderate, nodular and peripheral; in group III, a marked nodular infiltrate was seen both at the periphery and in the center of the tumour and in group IV, the lymphocytic infiltrate was diffuse and outnumbered the histiocytic tumour cells.

IMMUNOHISTOCHEMISTRY

For immunohistochemical studies, 3-µm sections were cut from each specimen. The expression of Vascular Endothelial Growth Factor (VEGF-A, JH121, NeoMarkers®, 1:100) and its receptor (VEGFR-2, Flk-1, Santa Cruz Biotechnology®, 1:100) were evaluated by streptavidin-biotin-peroxidase complex method, with a commercial detection system (Ultra Vision Detection System; Lab Vision Corporation, Fremont, USA) following the manufacturer's instructions. Antigen retrieval was carried out by microwave treatment (P=750W), for 20 minutes, in a citrate buffer (0,01M, pH6). Appropriated positive (canine angiosarcoma and mammary carcinoma) and negative controls were performed. Immunoreaction was visualized by incubation with 3,3-diaminobenzidine-tetrahydrochloride (DAB) at 0.05% with 0.01% H_2O_2 for 5 minutes. After a final washing in distilled water, the sections were counterstained with haematoxylin, dehydrated, cleared and mounted.

The assessment of VEGF expression was based on a semiquantitative analysis, according to the percentage of tumoral positive cells: - (negative), 0-5%; + (focal), 5-25%; ++ (multifocal), 25-50%; +++ (diffuse), > 51%).

STATISTICAL ANALYSIS

All analyses were performed using SPSSS statistical software (version 12.0; SPSS Inc., Chicago, USA). For studying categorical variables was performed a χ^2 test.

RESULTS

Most of the analyzed tumours were negative for VEGF-A (n=37; 74%) or had focal (n=6; 12%) or diffuse positivity (n=7; 14%). For VEGF-A differences between groups were statistically significant (p=0,002). Negative tumors generally belonged to histological groups I (fig.2) and II (fig. 3), both with low to moderate lymphoid infiltration, located at the periphery of the tumor.

The cases with a greater intensity and labelling extension belonged mainly to the histological group III (fig. 4), and also presented a large amount of lymphocytes, dispersed within the tumor.

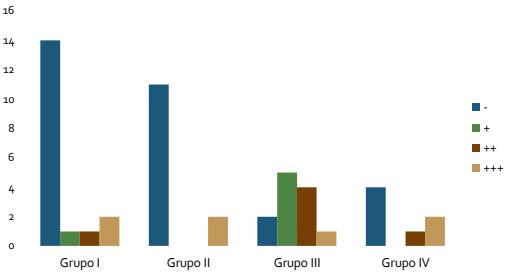

Figure 1: VEGF-A immunoexpression in HCC histological groups.

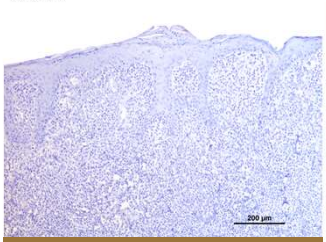

Figure 2: Group I canine cutaneous histiocytoma; tumour cells are negative for VEGF.

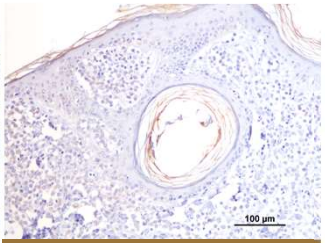

Figure 3: VEGF positive cells in a group II canine cutaneous histiocytoma.

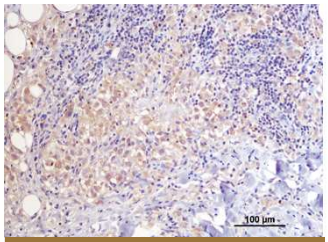

Figure 4: VEGF positive cells in a group III canine cutaneous histiocytoma.

VEGFR-2 immunoexpression was observed in the majority of cases (n=39; 78%); 7 tumors (17%) had focal positivity, 17 cases (43.5%) a multifocal labeling and 15 cases (38.5%) a diffuse labeling.

The differences obtained between the histological groups were statistically significant for VEGFR-2 (p<0,001). Thus, all the tumors of group I expressed VEGFR-2 with multifocal (n = 12) or diffuse (n = 6) labeling, figures 11 and 12. Tumors of group IV were mostly negative.

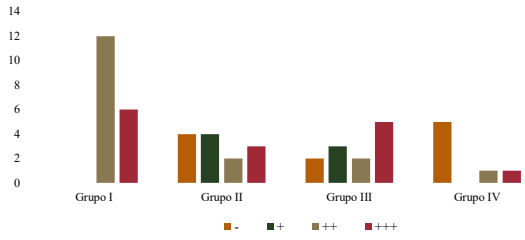

Figure 5: VEGFR-2 immunoexpression in HCC histological groups.

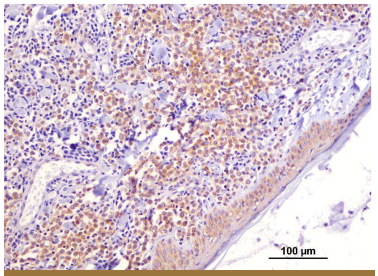

Figure 6: VEGFR-2 positive cells in a group I canine cutaneous histiocytoma with a diffuse labelling.

There was no statistically significant association between the immunohistochemical expression of VEGF-A and its receptor. However, in general, tumors of group I and II (in an early stage of regression) were negative to VEGF-A, but have a higher expression of VEGFR-2.

DISCUSSION AND CONCLUSIONS

Tumour angiogenesis is controlled by positive and negative modulators produced by the tumoral, stromal and inflammatory cells. Most of the tumour cells of CCH studied didn't produce VEGF or they produce it in inferior amounts to the detection threshold of the method used. Angiogenesis, essential for the growth and tumoural progression, seems to be committed in an initial phase of the histiocytoma development and can be decisive in the initiation of the regressive process. The increased cellular proliferation, without an appropriate blood supply, can be one of the first stimulus to cellular death by necrosis or apoptosis. The hypoxia, caused by absence of appropriate stimulus to the production of vessels, can unchain these two forms of cellular death, contributing to the regression of HCC. On the other hand, the immune response, associated to the regression of the histiocytoma would be probably committed with high levels of VEGF, since these are implicated in dendritic cells defective and in lymphocytes T correct development. The limited production of VEGF by tumoral cells of CCH by itself, doesn't justify the regression, but the interaction with many other processes, as for instance the immune response, can contribute to the regression of the CCH.

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