SHORT COMMUNICATION

Prognostic Significance and Immuno-Histochemical Expression of Vascular Endothelial Growth Factor in Canine Cutaneous Tumours

Veluchamy Kumar*, Senthilnayagam Hemalatha, Chidhambaram Balachandran, Ramasamy Thangathurai

Abstract

Vascular endothelial growth factor (VEGF) is the most thoroughly investigated in human oncology. It is the major angiogenic factor involved in physiological as well as pathological angiogenesis. Especially, in canine oncology vascular endothelial growth factor was a useful additional criterion for evaluating malignancy and growth potential in some tumours. But this angiogenic factor was still poorly investigated in veterinary oncology. The present work was undertaken to evaluate the expression of VEGF in the prognosis of cutaneous tumours in dogs. Fine needle aspiration cytology samples were collected from 103 dogs at Madras Veterinary College, Teaching Hospital, Chennai (India). VEGF expression was analyzed by immunohistochemistry on formalin fixed paraffin embedded sections of selected 28 canine cutaneous tumours. The VEGF immune expression was noticed in all types of cutaneous tumours in dogs. But the VEGF expression was more in all malignant tumours when compared to the benign tumours. Among the malignant tumours, metastasis and death cases showed higher expression. Besides, the tumours which showed high expression of VEGF showed higher malignant features than their counterparts. Thus, the study showed that there was prognostic significance in assessing tumour angiogenesis by independent VEGF expression in canine cutaneous tumours.

Key Words: Canine, Cutaneous tumours, Histopathology, Immunohistochemistry, VEGF expression

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Introduction

In dogs, approximately 30% of all neoplasms are reported to arise in the skin, in fact, they are the most and second-most frequently reported tumors in male and female dogs, respectively (Gamlem *et al.*, 2008). Angiogenesis is the process of new vessel formation and hallmark of tumour progression. Folkman *et al.* (1990) demonstrated that solid tumours cannot grow larger than 2-3 mm diameter without inducing their own blood supply. Tumour angiogenesis starts with the release of molecules by tumour cells that send signals to the surrounding normal host tissue, activates certain genes to make protein that encourage growth of new blood vessels (Yadav *et al.*, 2015). Some cells within small tumour change to an angiogenic phenotype by a phenomenon known as angiogenic switch. The molecular basis of this mechanism may be increased production of angiogenic factors or loss of angiogenesis inhibitors. VEGF is a secreted protein which promotes angiogenesis in tumours, chronic inflammation and healing of wounds. Up-regulation of VEGF expression has been associated with an increased risk of metastasis and poor prognosis in several human tumours including breast cancer (Schoeffner *et al.*, 2005; Pavlakis *et al.*, 2008). Nevertheless, there is still some controversy regarding the prognostic value of VEGF expression (Obermair *et al.*, 1997; Granato *et al.*, 2004). A few studies claim an association between VEGF over-expression and poor prognostic indicators e.g. histological grade and proliferative index (Dickinson and Karen, 2005; Dissi *et al.*, 2007). Hence, the present study was undertaken to evaluate the VEGF expression as a marker for angiogenesis in canine cutaneous tumours and assess the malignancy potential of the tumours.

Department of Veterinary Pathology, Veterinary College and Research Institute (VCRI) Tamil Nadu Veterinary and Animal Sciences University, Tirunelveli - 627 358, India

Corresponding Author: Dr. Veluchamy Kumar Department of Veterinary Pathology, Veterinary College and Research Institute (VCRI) Tamil Nadu Veterinary and Animal Sciences University, Tirunelveli - 627 358, India E-mail: drkumar2000@gmail.com

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Materials and Methods

Out of 103 dogs brought to Small Animal Clinic at Madras Veterinary College Teaching Hospital, Chennai (India) and during necropsy at Department of Veterinary Pathology, Madras Veterinary College, Chennai, twenty eight selected skin tumour tissue samples were collected by excisional biopsy. Epidemiological history like breed, age and sex and

clinical history like location, size, shape, weight and gross appearance of the skin tumours were recorded. Tissue samples were fixed in 10% neutral buffered formalin and routinely processed for histological examination. Paraffin embedded tissues were cut to 3-5 µ thickness and stained with H&E (Bancroft and Gamble, 2013).

Immunohistochemistry

Immunohistochemical staining was performed as per recommendation of the manufacturer (Bio-Genex USA) using super sensitive labeled poly HRP polymer method. Briefly, sections of 3-4 µm thick paraffin embedded tissue samples were collected on slides coated with polyL-lysine dried at 56ºC for 3-5 h. Paraffin sections were dewaxed by xylene, rehydrated and finally antigen retrieval done in 1M Tris EDTA buffer (pH-9) in a pressure cooker for 20 min. Blocking was done with power block solution. The sections were incubated with the primary antibody VEGF clone (JHC121) from Neo Marker at a dilution of 1:200; for negative control only diluent was applied for 2 h in a humidified chamber. After rinsing twice with 1M phosphate buffer saline + Tween 20, pH 7.4, the slides were sequentially treated with super enhancer and super sensitive horse radish Peroxidase (HRP) for 30 min. Diaminobenzidine (DAB) was used to develop a dark brown reaction product, counter stained with haematoxylin and washed with tap water, air dried and cleaned with xylene and mount with DPX. VEGF expression was evaluated at 40 x magnifications as per Restucci *et al.* (2002). At least 20 fields per tumour were examined, and a minimum of 100 neoplastic cells were counted. The VEGF intensity was scored as strong, moderate and mild. The VEGF distribution was scored as large (polarized) when the VEGF granules were restricted to the luminal pole of neoplastic cells and fine (cytoplasmic) when the VEGF granules were located diffusely in the cytoplasm.

Results and Discussion

The monoclonal antibody VEGF (JHC 121) immunolabelling was detected and is furnished in Table 1. In the present study of canine cutaneous tumours, VEGF expression was observed in the 28 tumour cases subjected to immunohistochemistry. Among the well differentiated squamous cell carcinoma (SCC) selected for study, non-metastasizing tumours expressed VEGF to the extent of 30 to 70% with moderate intensity and fine polarized cytoplasmic granules, while the metastasizing tumours (Fig. 1) showed expression to the extent of 80%

Table 1: Immunohistochemical evaluation of VEGF in canine cutaneous tumours

Type of tumour	No.	Staining Patterns		
		Positive of cells	Intensity	Granule Patterns
Squamous cell carcinoma	1	30%	Moderate	Fine (Cytoplasmic)
	2	50-70%	Moderate	Large (Polarized)
	3	80%	Strong	Large (Polarized)
	4	70-80%	Strong	Large (Polarized)
Basal cell carcinoma	1	10%	Mild	Fine (Cytoplasmic)
	2	10%	Mild	Fine (Cytoplasmic)
	3	30%	Mild to Moderate	Fine (Cytoplasmic)
Perianal adenoma	1	30%	Moderate	Fine (Cytoplasmic)
	2	40%	Moderate	Fine (Cytoplasmic)
Perianal adenocarcinoma	1	30%	Mild to Moderate	Fine (Cytoplasmic)
	2	40-50%	Moderate	Fine (Cytoplasmic)
	3	80%	Strong	Large (Cytoplasmic
	4	80%	Strong	Large (Cytoplasmic)
Sweat gland adenocarcinoma	1	40%	Moderate	Fine (Cytoplasmic)
	2	40 50%	Moderate	Fine (Polarized)
	3	70%	Strong	Large (Polarized)
Fibroma	1	10%	Mild	Fine (Cytoplasmic)
	2	10%	Mild	Fine (Cytoplasmic)
Fibrosarcoma	1	30%	Moderate	Fine (Cytoplasmic)
Liposarcoma	1	30%	Moderate	Large (Polarized)
	2	30%	Moderate	Fine (Cytoplasmic)
Mast cell tumour	1	30%	Mild	Fine (Cytoplasmic)
	2	40%	Moderate	Fine (Cytoplasmic)
	3	40%	Moderate to Strong	Large (Cytoplasmic)
	4	70%	Strong	Large (Cytoplasmic)
Histiocytoma	1	30%	Moderate	Fine (Cytoplasmic)
	2	50-70%	Moderate	Large (Polarized)
	3	70-80%	Strong	Large (Polarized)

(Fig. 2) and strong intensity with large polarized cytoplasmic granules. Maiolino *et al.* (2001) reported that VEGF expression was higher and more intense in invasive SCC in digits of dogs. Higher intensity of VEGF expression indicates the malignancy potential of the tumour, its ability to metastasize due to increased angiogenesis which might account for invasive or aggressive phenotype in canine SCC.

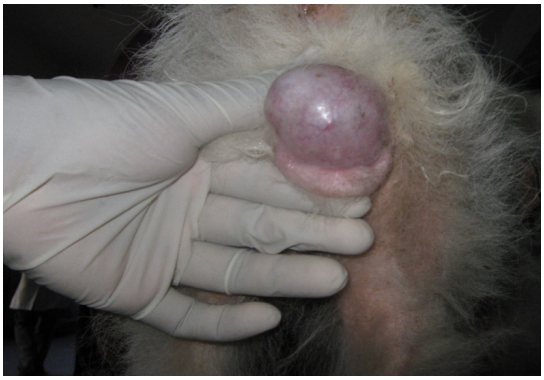

Fig 1: Squamous cell carcinoma –Vulva, Sptiz - 10 years

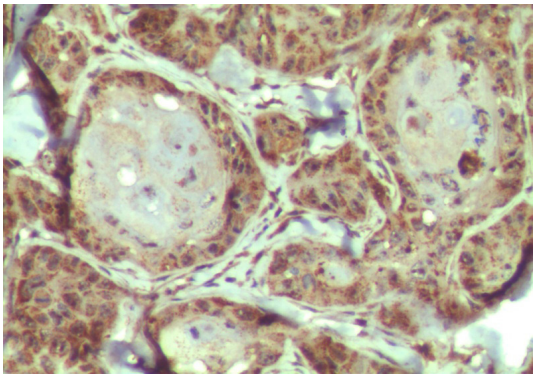

Fig 2: IHC-VEGF-Squamous cell carcinoma - Strong reaction 80% Brown coloured.Bar= 20 µm

In perianal adenoma, VEGF expression was mild when compared to the perianal adenocarcinoma which showed strong VEGF expression (Fig. 3, 4). There was no comparable literature in canine for these types of cutaneous tumours. The VEGF expression in sweat gland adenocarcinoma (Fig. 5) was 70% with strong intensity of staining (Fig. 6) in metastasizing tumour type than the non-metastasizing tumour.

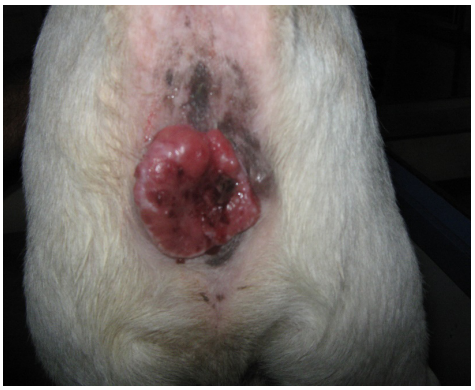

Fig 3: Perianal adenocarcinoma - Anal region, Lab - 10 years

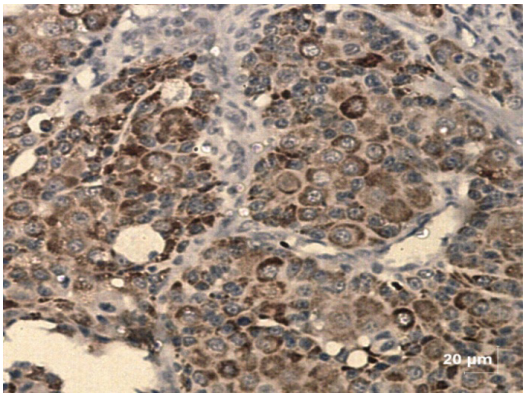

Fig 4: IHC - Perianal adenocarcinoma-Strong reaction 80%-Cytoplasmic staining with polarized granular pattern-brown coloured, Bar= 20 µm

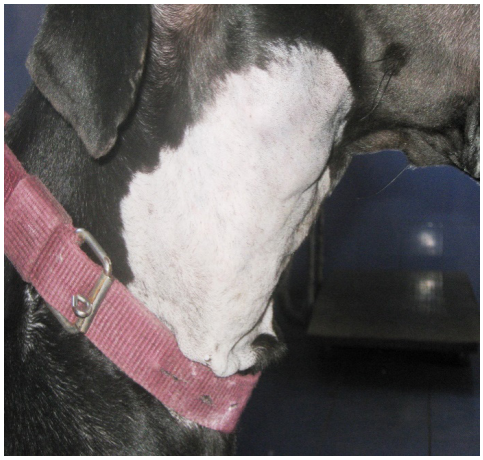

Fig 5: Sweat gland adenocarcinoma – Neck. Great Dane - 7 years

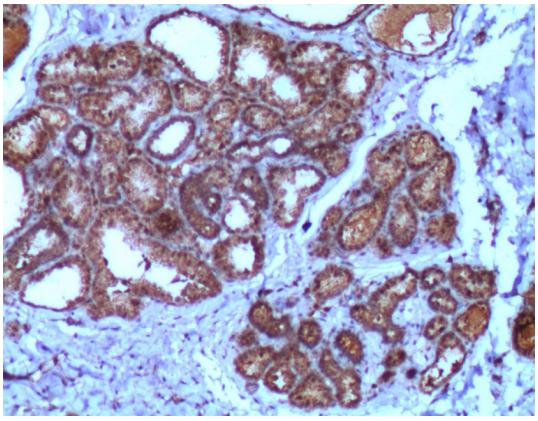

Fig 6: IHC-VEGF- Sweat gland adenocarcinoma - Cytoplasmic strong reaction 70% Brown coloured, Bar= 20 µm

When compared to the fibromas (10%), the fibrosarcoma expressed moderate intensity of VEGF staining (30%). The VEGF expression was moderate to strong intensity (30%) involving liposarcomas. These findings concurred with those of Luong *et al.* (2006), who reported more vascularized liposarcomas than other types of canine soft tissue sarcomas.

In four selected cases of Grade II mast cell tumours, the VEGF expression was strong with 70% extent among two tumour bearing dogs that died, while it was 30-40% with two cases mild to moderate intensity in the surviving

cases. In a recurrence case, the expression was 40% extent with moderate intensity of staining. In basal cell carcinomas (Fig. 7), the VEGF was distributed in 10-30 % of neoplastic cells (Fig. 8) and was mild to moderate in intensity, since canine basal cell carcinomas rarely metastasize although it is locally invasive. These findings concurred with the observation of Maiolino *et al*. (2000). In basal cell carcinomas, the VEGF was distributed in 10-30% of neoplastic cells and was mild to moderate in intensity, since canine basal cell carcinomas rarely metastasize although it is locally invasive. These findings concurred with the observation of Maiolino *et al*. (2000). In histiocytoma the VEGF expression was found to be 80% in a recurrent case when compared to 30 to 70% extent in other two cases. Luong *et al*. (2006) reported more vascularity in malignant fibrous histiocytoma. Millanta *et al*. (2002) studied the correlation of VEGF expression to overall survival in feline invasive mammary carcinomas. The VEGF expression significantly correlated with the overall survival time. The expression of VEGF in common canine cutaneous tumours studied showed a strong correlation between VEGF expression and tumour aggressiveness, intrinsic malignancy, growth potential and decreased survival.

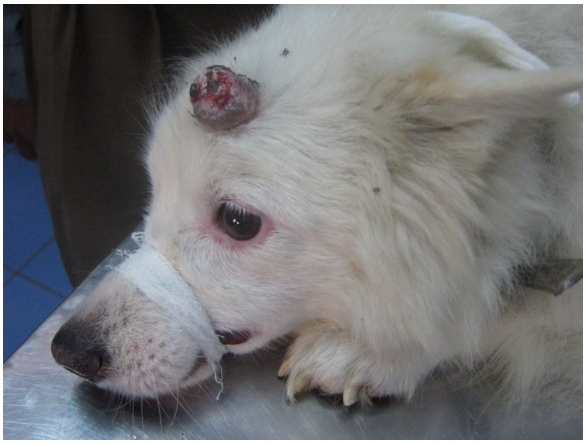

Fig 7: Basal cell carcinoma - Fore head, Spitz - 3.5 years

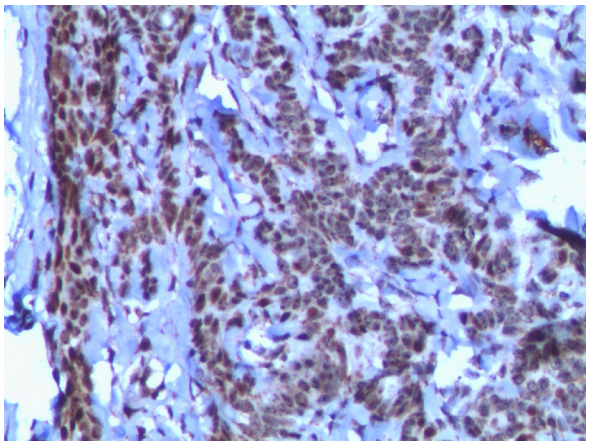

Fig 8: IHC-VEGF-BCC - Moderate reaction - 70%, Bar= 4 µm

Canine cutaneous tumours with increased expression of VEGF showed high malignancy features. The dogs with cutaneous tumours that showed strong, large and polarized expression of VEGF died within two months (Squamous cell carcinoma 41 days and Mast cell tumour 63 days) and recurrent cases died within 4 months (Perianal adenocarcinoma 72 days, histiocytoma 85 days and mast cell tumour 108 days).

Thus, VEGF expression could be correlated with malignancy, survival or recurrence and can be used as a prognostic marker for canine cutaneous tumours.

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