High Krüppel-like Factor 5 Expression is Associated with a Poor Prognosis in Dogs with Canine Mammary Carcinoma

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ABSTRACT

Canine mammary tumors are the most common neoplasms in female dogs. The Krüppel-like factor 5 (KLF5), a transcription factor, is involved in the pathobiology of cancer. The purpose of this study was to determine the expression of KLF5 and its clinical relevance in canine mammary tumor. One hundred forty-two canine mammary tumor specimens, comprising 75 carcinomas and 67 benign tumors were evaluated for KLF5 expression by immunohistochemistry and with the aid of Quick score. KLF5 expression was compared to various clinical and histologic parameters. The results indicated that high KLF5 level significantly correlated with large tumor size, high grade, advanced stages, and high Ki-67 index. Survival analysis also showed that high KLF5 expression was predictive of shorter survival in dogs with mammary carcinoma. This study revealed the pro-proliferation effect of KLF5 in canine mammary carcinoma and suggested that immunohistochemical analysis for KLF5 protein expression may contribute prognostic information.

INTRODUCTION

Mammary tumors are the second most frequently diagnosed neoplasms in dogs, next only to skin tumors.[1] They are the most common tumors in female dogs, comprising more than half of all neoplasms. Canine mammary tumors usually affect middle-age to older dogs, and the most common tumor cell type is adenocarcinoma.[2-4] The high similar epidemiologic, histologic, morphologic and clinical features suggest that dogs may be a promising model animal for comparative oncology.[5-14]

Krüppel-like factors (KLFs) are zinc finger-containing transcription factor highly conserved among mammals. KLFs collectively play important regulatory roles in

Table 1. *Differential expression of KLF5 expression in 142 cases of canine mammary tumor*

Histological classification	KLF5 expression (Quick score)				
	Negative (0)	Low (1,2)	Moderate (3,4)	High (5-7)	total
Benign tumor	33(49.3%)	23(34.3%)	10(14.9%)	1(1.5%)	67
Carcinoma	2(2.7%)	1(1.3%)	26(34.7%)	46(61.3%)	75

diverse biological processes such as growth, development, differentiation, and apoptosis. Many KLFs are also involved in the pathobiology of cancer.[15-17]

KLF5 has been well documented to promote cell proliferation. KLF5 expression correlates with that of HER2 and the proliferation marker, Ki67, in human breast cancer.[18] It has also been reported that breast cancer patients with higher expression levels of KLF5 have shorter disease-free survival and overall survival than patients with lower KLF5 expression.[18] Unlike the pro-proliferative role described above, frequently hemizygous deletion and loss of expression of KLF5 has also been described in breast tumors, indicating a possible tumor suppressive-like role.[19]

In this report, we aimed to examine the presence of KLF5 and evaluate its clinical significance in canine mammary tumor by measuring the expression of KLF5 and correlate the expression with clinicopathologic data and survival outcome.

MATERIALS AND METHODS

Sample selection

Archive tissue blocks of 142 cases of canine mammary tumor diagnosed between January 2003 and April 2008 were obtained from the School of Veterinary Medicine, National Taiwan University, Taiwan. Seventy-five of the 142 mammary tumors were histologically confirmed as carcinomas and 67 were benign tumors. Of the 75 cases of carcinoma, 35 were simple carcinomas, 35 were complex carcinomas, and 5 cases were carcinoma in benign tumor.

Tumor diagnoses were made according to the World Health Organization International Histological Classification of Tumors of Domestic Animals.20 Histological grading was determined on archive HE-stained sections based on tubule formation, nuclear pleomorphism, and mitotic counts, with feature scored 1 to 3 points then added to obtain the tumor grade.[21,22] Tumor size measured maximum diameter and was classified according to the WHO Clinical Staging System TNM as T1 (<3 cm), T2 (3–5 cm) and T3 (>5 cm).[23]

Immunohistochemical staining

Paraffin-embedded canine mammary tumor tissue sections (4-μm) mounted on poly-L-

Figure 1. *KLF5 expression as evaluated by Quick score. (A) High nuclear KLF5 expression with quick score of 5-7, (B) moderate expression with score of 3 or 4, (C) low expression with score of 1 or 2, and (D) no KLF5 expression with score of 0 (×400).*

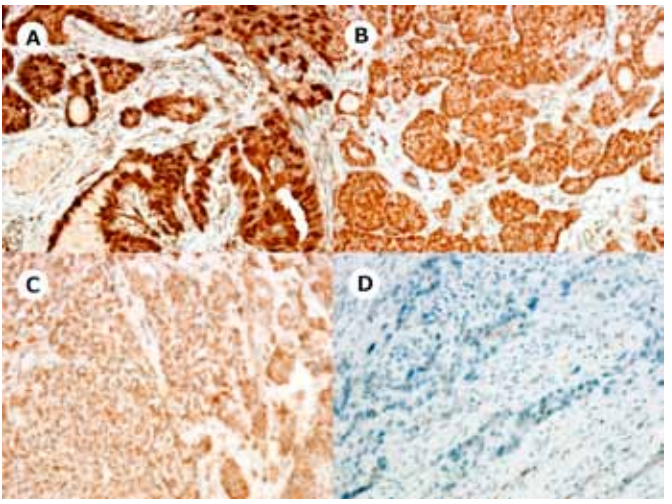
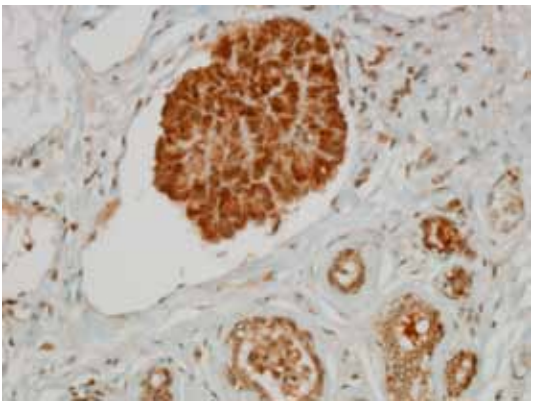


Table 2. *Correlation of nuclear KLF5 expression with clinicopathologic parameters in canine mammary carcinoma*

		KLF5 expression				N	P
		<5		≥5			
Age(SD)		11.5 (2.8)		12.4 (2.0)			0.109
Ovariohysterectomy							
	No	25	(86.2%)	34	(73.9%)	59	0.206
	Yes	4	(13.8%)	12	(26.1%)	16	
Tumor Size							
	T1	17	(58.6%)	9	(19.6%)	26	0.002
	T2	7	(24.1%)	18	(39.1%)	25	
	T3	5	(17.2%)	19	(41.3%)	24	
Grade							
	I	14	(48.3%)	3	(6.5%)	17	<0.001
	II	11	(37.9%)	25	(54.3%)	36	
	III	4	(13.8%)	18	(39.1%)	22	
Location of affected gland							
	cranial	11	(37.9%)	16	(34.8%)	27	0.782
	caudal	18	(62.1%)	30	(65.2%)	48	
Stage							
	I	16	(55.2%)	4	(8.7%)	20	<0.001
	II	6	(20.7%)	12	(26.1%)	18	
	III	3	(10.3%)	8	(17.4%)	11	
	IV	3	(10.3%)	16	(34.8%)	19	
	V	1	(3.4%)	6	(13.0%)	7	
ER							
	Negative	17	(58.6%)	22	(47.8%)	39	0.362
	Positive	12	(41.4%)	24	(52.2%)	36	
PR							
	Negative	3	(10.3%)	8	(17.4%)	11	0.513
	Positive	26	(89.7%)	38	(82.6%)	64	
HER2 overexpression							
	Negative	24	(82.8%)	34	(73.9%)	58	0.373
	Positive	5	(17.2%)	12	(26.1%)	17	
Ki-67							
	<10%	5	(17.2%)	2	(4.3%)	7	0.015
	10-50%	14	(48.3%)	14	(30.4%)	28	
	51-100%	10	(34.5%)	30	(65.2%)	40	

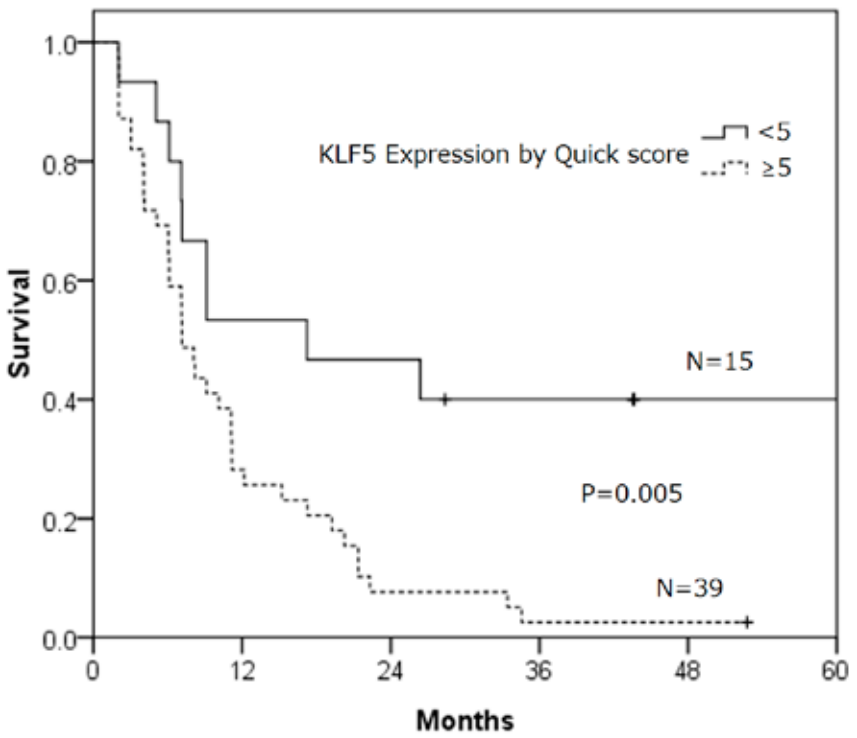
lysine-coated slides, were dewaxed in xylene and hydrated by passage through graded alcohols. Endogenous paroxidase was quenched with methanol and 3% hydrogen peroxide. Slides were then immersed in 10mM citrate buffer (pH 6.0) and heated at 100°C for 20 min. Samples were incubated with anti-KLF5 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at 1:60 dilution for 1 hour at room temperature, followed by washing thoroughly three times with PBS. Bound antibodies were detected using the EnVision Detection Systems Peroxidase/DAB, Rabbit / Mouse kit (Dako, Glostrup, Denmark). The sections were counterstained with Gill Hematoxylin Solution II (MERCK, Darmstadt, Germany). Paraffin-embedded human breast cancer tissues that showed homogeneous KLF5 staining were used as positive controls. Negative controls had the primary antibody replaced by PBS.

Figure 2. *Strong nuclear KLF5 staining outlines tumor emboli in a representative canine mammary carcinoma case (400X).*



Quantification of KLF5 expression was made using Quick score system.[24,25] The intensity of the immunohistochemical reaction as viewed under the light microscope was

Figure 3. *The Kaplan-Meier plot with survival as function of KLF5 expression.*



recorded as 0, 1, 2, and 3 for negative, weak, moderate, and strong staining, respectively. The percentage of cells staining positively at any intensity was scored as follows: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100%, compared with the total tumor cells. The percentage and intensity scores were added together to obtain a total score ranging from 0 to 7. The scoring was performed by 2 researchers under light microscopy.

Staining of ER (anti-ER antibody at 1:35 dilution, Dako, Glostrup, Denmark), PR (anti-PR monoclonal at 1:200 dilution, Thermo Scientific, Fremont, CA, USA), HER2 (anti-HER2 antibody at 1:400 dilution, Dako, Glostrup, Denmark), and Ki-67 (anti-Ki-67 antibody at 1:100 dilution, Thermo Scientific, Fremont, CA, USA) was also carried out with the similar procedures described above. Nuclear staining of ER, and PR in more than 10% of the cells were considered positive.13 HER2 expression was evaluated according to the American Society of Clinical Oncology/College of American Pathologists recommendations.[26] A score of 3+ indicated HER2 positivity.

Statistical analysis

Correlations between KLF5 expression and clinicopathologic parameters were evaluated by Pearson's correlation analysis. Survival analysis was performed using Kaplan-Meier method and with the Cochran-Mantel-Haenszel test to determine statistical difference. Survival was defined as the time between the date of diagnosis and date of cancer-causing death or last follow-up. Follow-up data was collected up to August 2010. Subjects still alive at the end of the study were censored at the date of last follow-up. Cases that lacked complete survival information were excluded from survival analysis.

RESULTS

Immunohistochemical staining of 142 dogs with mammary tumors were sorted into four groups according to KLF5 expression level: High nuclear KLF5 expression with Quick score of 5-7, moderate expression with score of 3 or 4, low expression with score of 1 or 2, and negative KLF5 expression with score of 0 (Fig. 1). High KLF5 expression occurred preferentially in carcinomas as it is noted in 46 of the 75 (61.3%) cases of carcinoma. In contrast, High KLF5 expression was detected in only one of the 67 (1.5%) benign tumors (Table 1). Moreover, KLF5 was high expressed only in the tumor cells and not the adjacent normal tissues in mammary carcinomas (Fig. 2). Table 2 summarizes the association of differential KLF5 expression with various clinical and histopathologic variables in canine mammary carcinoma. High expression of KLF5 was significantly linked with larger tumor size ($P = 0.002$), higher histological grade ($P < 0.001$), more advanced stage ($P < 0.001$), and high Ki-67 expression level ($P = 0.015$). Figure 3 depicts the survival of the dogs with mammary carcinoma according to KLF5 expression status. The Kaplan-Meier survival plot showed that dogs with high KLF5 expression (Quick score ≥ 5) had a significantly shorter survival as compared with ones with moderate/low/negative KLF5 expression (Quick score <5) ($P = 0.005$).

DISCUSSION

The spontaneous mammary tumors in dogs may offer an opportunity as models for human breast cancer biology and therapeutics.27 Basic and clinical research studying mammary gland tumors in dogs has the potential to benefit both dogs and women affected with this disease.

The role of KLF5 in the pathogenesis of breast cancer has yet to be firmly established, with some reports showed that it promotes breast tumor formation while others claimed a possible tumor suppressive role.[17]

We studied the expression of KLF5 and found differential expression among the 142 cases of canine mammary tumor. High level of KLF5 expression occurred more frequently in cases with mammary carcinoma and was preferentially present in the carcinoma cells as illustrated in Figure 2. High KLF5 expression was significantly associated with unfavorable clinical and histologic

features such as large tumor, high grade, and advanced stages.

Ki-67 is present at low levels in quiescent cells but is increased in proliferating cells, especially in the G2, M, and latter half of the S phase. Elevated Ki-67 expression is frequently seen in various cancers[28-35] and is associated with poor clinical outcome in patients with breast cancer[36]. Ki-67 immunohistochemistry has been shown to provide a reliable assessment of the growth fraction of neoplastic tissues.[37] Higher Ki-67 is generally associated with the greater the risk of an adverse outcome. For breast cancer, prognosis is considered to be favorable with Ki-67 <10%. Our results indicated that expression of KLF5 coincides with that of Ki67 in canine mammary carcinoma.

Although it is yet to be firmly established, KLF5 protein expression has been shown to have prognostic value in human breast carcinomas and to correlate with HER2 positivity. This study suggested that KLF5 expression was not associated with ER, PR, or HER2 status in canine mammary carcinoma.

Our survival analysis further indicated that high KLF5 expression is a prognostic marker for survival of dogs with mammary carcinoma. This prognostic significance of KLF5 may be explained by its pro-proliferative effect on tumor cells.

Taken together and given the significant similarities between human breast cancer and canine mammary carcinoma, the present study supported the promoting effect of KLF5 on cell proliferation. This study is the first to demonstrate the pro-proliferative role of KLF5 and its prognostic value for survival in canine mammary carcinoma.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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