

Paper Submission Instructions (OIE/15th FAVA 27-29 October 2008)

In general

- Papers must be in Microsoft Word format (Microsoft Word version 2000 or higher), written in Times New Roman 10 pt on A4 papers (8.5 inches x 11 inches).
- The mandatory template will give the following margins: top 2.5 cm, bottom 2.3 cm, left 1.6 cm, right 1.6 cm.
- The template will divide the entire text into two columns. The space between columns will be 1.0 cm.
- The right margin of the text will be justified.
- The entire paper can not exceed two pages.
- All texts are in single spaced, including subheadings, tables and figures.
- Do not include page numbers or line numbers.

Title (Bold in Times New Roman 14 pt) must be completed within 2 lines.

Authors

- Use 10 pt Times New Roman, initial(s) followed by lastname of all authors in regular typing.
- The name of the presenting author should be in bold and underlined; the corresponding author is emphasized by an asterisk.

Affiliations

Keywords (not more than 5 keywords)

Subheadings

- The paper should contain following subheadings: Introduction, Materials and Methods, Results, Discussion or Results and Discussion, References and Acknowledgement. If references and/or acknowledgement are not applied, omit these subheadings.
- Subheadings should be in bold. Do not use capital letters.
- Each subheading should be placed on a single line without any body text using a double space between the previous text and the subheading.

Body text

- The text should be divided into two columns and written immediately beneath the subheading (no blank line under the subheading).
- The paper should be understandable on its own.
- The text should state clearly the objective, methods, results, discussions and conclusions of the study.
- Leave single space between paragraphs. Do not use indentations.
- Use standard abbreviations. Place a special or unusual abbreviation in parentheses after the complete word the first time it is used.
- Use a dot in front of decimals (example: 0.23). Use a comma in numbers greater than 999 (example: 2,358).
- If sub-subheadings are used, they should be written in italics and followed by a colon and text in the same line (example: *Blood samples*: On day 7, blood samples were taken from....). Leave a blank line above sub-subheadings.
- References should be numbered in the order they are mentioned in the text. Cite references in the text by placing the number in parentheses, for example: (1)

Introduction: The problem(s) under investigation or proposed hypothesis.

Materials & Methods: Experimental methods used (including microorganisms and/or samples).

Results: In summary form, with sufficient quantitative data and statistical tests. Summary such as “to be completed” or statements as such are not acceptable.

Discussion: Summary of findings that are supported by the results (statement such as “The results will be presented and discussed” will not be accepted).

References

- In the list of references, references should be written and numbered in the order they are mentioned in the text.
- Do not leave blank lines between the references.
- The titles of the references should be omitted.
- In case of more than two authors, use only the lastname of the first author, followed by et al.
- The year of the publication should follow the authors' lastnames .
- Use abbreviations on names of journals.

Tables and figures

- Figures and tables must not be wider than a text column and should be suitable for printing in black and white.
- Colored photos may be used but should be suitable for printing in black and white.
- Table headings should be written above the table.
- Figure headings should be written below the figure.
- Write “**Table 1**” and “**Figure 1**” in bold. The rest of each table/figure heading should be in regular typing.
- Limit the number of lines in tables. Avoid vertical lines if possible.

C-erbB2 Oncogene Expression as a Prognostic Factor in Canine Mammary Adenocarcinomas

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Introduction

Canine mammary tumors rank the second in frequency, following skin neoplasm. Recent reports indicated the application of TNM system, histopathological characteristics, lymph nodes metastasis and proliferative markers for precise diagnosis and prognostic values. The common use of proliferative markers in malignant tumors are thymidine-labeling index, Ki-67, proliferating cell nuclear antigen (PCNA) and argyrophilic nucleolar organizer regions (AgNORs). PCNA is a 36 kDa nuclear protein associated with cell cycle regulated nuclear protein and co-factor of polymerase delta enzyme (2). PCNA index indicated proliferative activity of benign and malignant canine mammary tumors and was related to the malignancy histopathological type (10).

C-erbB2 oncogene; synonym neu/HER2, is a 185 kDa glycoprotein located in cytoplasmic cell membrane and plays a major role in intrinsic protein tyrosine kinase activity (6,7). Overexpression of *c-erbB2* oncogene was reported in human breast and ovarian cancers. The *erbB2* oncogene amplification related with poor prognosis, relapsing survival time, estrogen or progesterone hormonal status metastasis and chemotherapy response in human mammary adenocarcinomas (6). The prognostic value of *c-erbB2* in canine mammary tumors was reported in various histologic types (1, 11) but there are no supported data on the clinical prognostic factor as compared to human cases. The aim of this study is to determine the relationship between the *c-erbB2* oncogene expression and canine mammary adenocarcinomas based on histopathological grade and PCNA proliferative marker index.

Materials and Methods

Specimens: 96 biopsy specimens, including mammary tumor mass and regional lymph node, were collected from canine patients presenting at the Small Animal Hospital, Chulalongkon University from 1997 to 2001. Clinical data based on TNM system were noted. Representative tissues were fixed in 10% neutral buffered formalin for a routine histologic procedure with HE staining. Other special stainings (PAS, MT) and immunohistochemistry were applied for a making of definite diagnosis. The histopathological diagnosis was based on WHO classification (5). The histological malignancy grade was classified into grade 0, I and II, according to cytoplasm and nuclear characteristics of tumor cells, cellular arrangement and tissue invasiveness (4). The nuclear grade was defined as grade 1 (poorly differentiated), grade 2 (moderately differentiated) and grade 3 (well differentiated) (4).

Immunohistochemistry: The avidin biotin complex method was performed both PCNA and *c-erbB2* immunostaining on formalized and paraffinized sections. Briefly, for PCNA immunostaining, the sections were treated in distilled water with microwave heat technique (900w, medium heat, 5 mins twice) and incubated with monoclonal mouse anti-PCNA antibody (dilution 1:200, Dako, Denmark). The number of positive cells per 1500 examined was expressed in percentage. For *c-erbB2* immunostaining, the same serial sections were treated in citrate buffer, pH 6.0 by microwave heat (900w, medium heat, 5 mins twice) and incubated with rabbit polyclonal anti-human *c-erbB2* oncoprotein (dilution 1:200, Dako, Denmark, 11). Human invasive ductal carcinoma and normal canine mammary tissue were used as positive and negative control, respectively. *C-erbB2* expression was presented as percentage of positive cases and 0, +1, ++ and +++ (8).

1 cm

Chi-square, Fisher exact test and logistic regression analysis with p -value ≤ 0.05 were the methods selected for the statistical analysis.

Results and Discussion

The pattern of membranous and cytoplasmic *c-erbB2* expression was observed in glandular and ductal tumor cells especially in case of squamous metaplasia in 74%(71.96) of canine mammary adenocarcinomas (Table 1, Fig. 1). The loss of *c-erbB2* expression in normal mammary tissue proved the notion that *c-erbB2* involved in the transformation of canine mammary tumors (11). *C-erbB2* protein did not have statistical differences on histopathological characteristics based on WHO classification, malignancy grading and nuclear differentiation. The percentage of positive cases in each histopathological type showed similar proportion as well as malignancy grade. It is interesting that *c-erbB2* expression was detected 80% in well differentiated nuclear grade and decreased in moderately and poorly differentiated nuclear grade. The *c-erbB2* expression was accepted to use as prognostic factor of individual tumor type in human as ductal carcinoma *in situ* and invasive ductal carcinoma (3) without significant relation. The *c-erbB2* oncogene product indicated the malignancy behavior of mammary cancer and related with lymphatic and blood vessel metastasis and estrogen or progesterone receptor in human case (8). The PCNA index has statistical difference between histopathological type ($p=0.001$) and the index increased when the tumor became malignancy. PCNA can be served

as a prognosis indicator in canine mammary adenocarcinomas (Table 2) (9).

The *c-erbB2* did not significantly difference with PCNA index suggesting that PCNA index is an independent prognostic indicator in canine mammary adenocarcinomas. From this study suggested that the application of *c-erbB-2* oncogene in the prognosis of canine mammary adenocarcinomas is suitable in each histopathological type as previously reported in human.

Acknowledgement

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References

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Table 1 Results of *c-erbB2* expression in canine mammary adenocarcinomas (n=96)

Tumor characteristics	<i>C-erbB-2</i> expression			
	Positive	+	++	+++
1. Histologic type				
Simple tubular ACC	41(73.2%)	4(7.1%)	6(10.7%)	20(35.7%)
Complex tubular ACC	7(70%)	-	3(30%)	2(20%)
Simple papillary ACC	5(83.3%)	-	2(33.3%)	2(33.3%)
Cystic papillary ACC	1(100%)	-	-	1(100%)
Solid carcinoma	17(77.3%)	5(22.7%)	2(9.1%)	4(18.2%)
Secretory cell carcinoma	-	-	-	-
	71(74%)			
2. Malignancy grading				
Grade 0	16 (80%)	-	5(25%)	6(30%)
Grade I	38 (66.7%)	7(12.3%)	6(10.5%)	14(24.6%)
Grade II	15 (88.2%)	2(11.8%)	2(11.8%)	7(41.2%)
3. Nuclear grade				
Well	33 (82.5%)	2(5%)	8(20%)	15(37.5%)
Moderately	33 (67.4%)	6(12.2%)	4(8.2%)	12(24.5%)
Poorly	5 (60%)	1(14.3%)	1(14.3%)	2(28.6%)

*ACC: adenocarcinomas

Table 2 Results of PCNA index in positive cases of canine mammary adenocarcinomas (mean±SD)

1. <i>C-erbB2</i> expression	PCNA index(%)
Positive	40.81±18.77
+	40.43±19.45
++	44.98±21.40
+++	42.88±18.98
Negative	36.25±17.18
2. Malignancy grading	
Grade 0	28.91±11.44
Grade I	41.10±18.94
Grade II	49.58±17.77

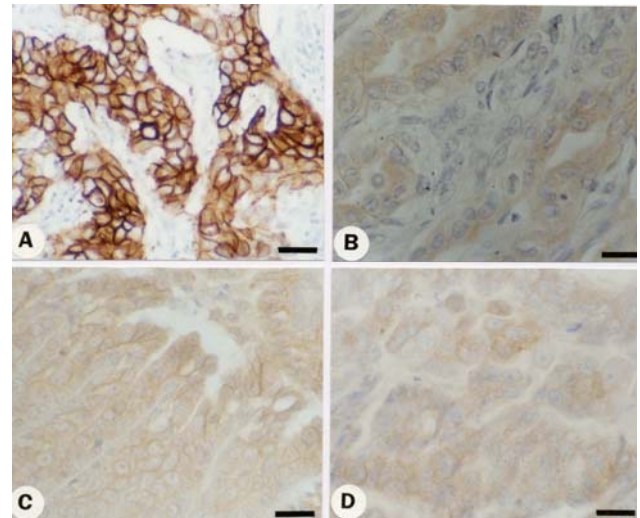


Figure 1: *C-erbB2* expression in canine mammary adenocarcinomas, IHC; DAB counterstained with Meyer's hematoxylin, bar =25 µm

- C-erbB2* expression in human invasive ductal carcinoma (positive control, bar =50 µm)
- C-erbB2* expression in simple tubular adenocarcinoma (D6934M)
- C-erbB2* expression in simple papillary adenocarcinoma (D8703N)
- C-erbB2* expression in solid carcinoma (R1913N)

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