HER-2/neu Protein Expression in Canine Mammary Adenocarcinoma

Hai-Jie Yang, Sun Hee Do[1], Dong-Wei Yuan, Il-Hwa Hong, Mi-Ran Ki, Jin-Kyu Park, Moon-Jung Goo, Hye-Rim Lee, Kyung-Sook Hong, Ok-Kyung Hwang, Jung-Youn Han, Ho-Yong Park[2], Sung-Eun Yoo[3] and Kyu-Shik Jeong*

Department of Veterinary Pathology, Collage of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Republic of Korea
[1]*Department of Clinical Pathology, College of Veterinary Medicine, Konkuk University, Seoul, 143-701, Republic of Korea*
[2]*Insect Biotec Co., Ltd., Daejeon, 305-815, Republic of Korea*
[3]*KOREA Research Institute of Chemical Technology, Daejeon, Republic of Korea*

Received December 7, 2007 / Accepted December 27, 2007

In this study to evaluate the involvement of EGFR, HER-2/neu and ALCAM (CD166) oncogene products in canine mammary neoplastic lesions, sections of archived paraffin-embedded samples of 49 mammary tumors were analyzed immunohistochemically using antibodies against human EGFR and HER-2/neu and ALCAM. These 49 tumors were divided into 2 groups: 22 benign (19 adenoma, 3 benign mixed tumors) and 27 malignant tumors (2 simple adenocarcinomas, 5 complex adenocarcinomas, 3 solid carcinoma, 5 sclerosing carcinoma, 8 malignant mixed tumors and 4 malignant myoepithelioma). As a result of immunostaining, 31.8% (7/22) of the benign tumors and 29.6% (8/27) of the malignant tumors expressed the HER-2/neu oncogene product, EGFR expression was detected in 27.3% (6/22) of benign tumors and in 22.2% (6/27) of the malignant tumors. ALCAM expression was detected in 40.9% (9/22) of benign tumors and in 7.4% (2/27) of the malignant tumors. These results suggest that some of the biological and morphological characteristics of the tumor are associated with canine mammary gland tumors, as also reported for human breast cancer, the possibility of using anti-HER-2/neu antibodies in the treatment of canine mammary tumors.

Key words : Human epidermal growth factor receptor (HER-2/neu), epidermal growth factor receptor (EGFR), activated leukocyte cell adhesion molecule (ALCAM), canine, mammary, adenocarcinoma

Introduction

The mammary gland is one of the most common sites of tumor development in dogs and cats [30]. Approximately 50% of canine mammary tumors are malignant and the most common malignant tumors are solid carcinomas and adenocarcinomas [10]. Most tumors occur in dogs between 8 and 10 years of age. Mammary cancer occurs much less frequently in cats than in dogs, but when it does occur it is often malignant and difficult to treat. Mammary cancer is likely to strike 1 in 4000 cats. While this is about half the rate as in dogs, when cats develop mammary tumor it is often fatal. Any adult female cat can develop mammary cancer, but the median age is usually 10-14 years. Eighty-five percent of mammary tumors in cats are malignant adenocarcinomas. Other mammary tumors in cats include duct papillomas, sarcomas and adenomas [30]. Recently, there has been considerable interest in the role of oncogenes and tumor suppressor genes in the etiology and progression of many carcinomas, as studies on these genes may provide useful information on the course of malignant diseases and help with treatment selection [12,17].

A number of proto-oncogenes and oncogenes have been investigated in breast cancer due to their relevant roles in breast carcinogenesis, as well as for their prognostic value. Proto-oncogenes are normal genes that are usually responsible for the regulation of cell proliferation, but in mutated type promote neoplastic transformation. c-erbB-2, c-RAS are examples of some oncogenes involved in mammary carcinogenesis.

In recent years, there has been a dramatic increase in our understanding of the biology of cancer. An area that has received considerable interest by both basic scientists and clinicians is that of prognostic markers in breast cancer. The HER-2/neu oncogene (also called c-erbB-2) has generated excitement and many publications because it is one of the first examples in breast cancer in which molec-

***Corresponding author**
Tel : +82-53-950-5975, Fax : +82-53-950-5955
E-mail : jeongks@knu.ac.kr

ular biology has been applied clinically [27,28].

HER-2/neu overexpression has been detected in several solid tumor malignancies, but research has focused particularly on its role in breast cancer because of the high prevalence of the disease [22]. Slamon et al [29] first described the observation of HER-2/neu oncogene amplification in human breast cancer. A number of studies have now confirmed that the HER-2/neu oncogene is either amplified or its product overexpressed in 10% to 40% of primary human breast cancers [2,29].

The demonstration of a high frequency of c-erbB-2 overexpression in in situ ductal carcinomas of the breast suggests that it could be an early event in breast carcinogenesis in humans [5,23], c-erbB-2 overexpression has also been detected in canine mammary gland tumors [24].

Epidermal growth factor receptor (EGFR) is the expression product of oncogene c-erbB-1. It plays an important role in growth and differentiation of tumor cells and it improves growth and metastasis of tumor [31]. The is a 170 kDa cell surface transmembrane receptor with tyrosine kinase activity [15], which has a number of ligands including epidermal growth factor (EGF) and transforming growth factoralpha (TGF-a).

Epidermal growth factor receptor (EGFR) is expressed in all epithelial and many normal mesenchymal cells and has a wide range of function. In resting, non-transformed cells EGFR signaling is tightly controlled. In tumors, oncogenic activation of this pathway occurs as a result of mutation, overexpression, structural arrangements, and release from its normal monomer. Stimulation of EGFR pathway leads to enhancement of cellular proliferation, up-regulated expression of vascular endothelial growth factors, prevention of apoptosis, and invasion. Many different types of carcinoma have been shown to express EGFR including renal cell [26], and breast [14]. Studies have shown that tumor EGFR expression is an indicator of poor prognosis, decreased survival, and increased metastases in several tumor types [25]. The significance of tumor EGFR expression in regard to EGFR inhibitor therapy remains to be determined.

Activated leukocyte cell adhesion molecule (ALCAM) is a glycoprotein of the immunoglobulin super-family. ALCAM has been identified in multiple species and has different names depending on the species and laboratory that described it: human melanoma metastasis clone D [9], mouse/human CD166 [6]. ALCAM has been shown to be involved in capillary tube formation [21] and in vessel invasion into cartilage *in vitro* [4].

In this study, we were to examine and evaluate the involvement of HER-2/neu, EGFR and ALCAM in canine spontaneous mammary gland adenocarcinoma.

Materials and Methods

Case selection

Tissue biopsy specimens of 49 canine mammary gland tumors taken during the period 2002 and 2005 were collected from the archives of the department of veterinary pathology, university of kyungpook national, Korea. Clinical history data, including the dog's ages, tumor sizes and the clinical stages accessible according to the staging system of rosen and oberman were recorded [25]. Histopathologic examination, diagnosis and tumor classification were carried out according to the World Health Organization criteria for histologic typing of canine mammary tumors.

Histopathology and immunohistochemistry

Mammary tumor tissues were fixed immediately in 10% neutral buffered formalin for light microscopy, processed routinely and embedded in paraffin. Sections were cut to 4 um in thickness. The sections were stained with hematoxylin and eosin (H&E). For immunohistochemistry, sections of mammary tumor tissue were deparaffinized in xylene, rehydrated in graded alcohol series, incubated in a solution of 3% hydrogen peroxide in methanol for 30 minutes and microwaved at 750 W for 10 minutes in 10 mmol/L citrate buffer, pH 6.0. Tissue sections were washed with phosphate-buffered saline (PBS), and then immunostained with primary antibody. The primary antibodies used were monoclonal antibody HER-2/neu (Clone CB11, BioGenex, San Ramon, CA), EGFR (Santa Cruz Biotechnology, Inc., California, USA), ALCAM (Novocastra Laboratories Ltd., Newcastle-upon-Tyne, UK) respectively. The antigen-antibody complex was visualized by an avidin-biotin-peroxidase complex (ABC) solution using an ABC kit (Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (Zymed Laboratories Inc., San Francisco, CA, USA). Mammary tumor tissue sections were then rinsed in distilled water and counterstained with Mayer's hematoxylin. The primary antibodies used were: monoclonal antibody HER-2/neu at a dilution of 1:100,

Table 1. Immunohistochemical evaluation

Score	Assessment of HER-2/neu protein overexpression	Localization
0	Negative	No staining is observed, or membrane staining in less than 10% of the tumor cells
1 +	Negative	A faint barely perceptive membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of membrane
2 +	Positive	A weak to moderate complete membrane staining. Staining is observed in 10% of the tumor cells
3 +	Positive	A strong complete membrane staining is observed in more than 10% of the tumor cells

EGFR at a dilution of 1:300, mouse monoclonal antibody ALCAM at a dilution of 1:50. For negative control, the primary antibody was replaced by phosphate-buffered saline.

Immunohistochemical evaluation

Immunohistochemical evaluation are shown in Table 1.

Results

Forty-nine canine mammary tumors were divided into 2 group comprising 22 benign mammary lesions and 27 malignant tumors. The mean ages of 49 dogs were 8.58 (range, 2 to 15) years. The mean ages at diagnosis of the benign and malignant groups were 8.29 (range, 3 to 14) years and 8.81 (range, 2 to 15) years, respectively. The clinical data are summarized in Table 2.

The histological classifications of the 22 benign mammary tumors were 19 adenomas, 3 benign mixed tumors. The 27 malignant tumors were categorized as 15 adenocarcinomas which were subdivided into 2 simple papillary types, 5 complex types, 3 solid carcinoma and 5 sclerosing carcinoma, 8 malignant mixed tumors and 4 malignant myoepithelioma. The histological data are presented in Table 3.

The immunohistochemical results are summarized in Table 4. Positive HER-2/neu oncogene immunoreactivity was observed in 30.6% (15/49) of the mammary tumors examined. This cell membrane staining pattern was observed exclusively in neoplastic cells, and positive staining in most cases was distributed uniformly throughout the neoplastic cell population, although the immunostaining intensities of cells of individual tumors showed variations. 31.8% (7/22) (5 adenomas and 2 benign mixed tumors) of the benign group showed positive immunostaining (Fig. 1),

Table 2. Clinical features of 49 canine mammary tumors

	Benign tumor (total 22 cases)	Malignant tumor (total 27 cases)
Age (years) 2~15 (mean=8.58)	3~14 (mean=8.29)	2~15 (mean=8.81)
<10 years old	14/22 (63.6%)	13/27 (48.1%)
≥10 years old	8/22 (36.4%)	14/27 (51.9%)

Table 3. Histological typing of 49 canine mammary tumors

Mammary tumor	Number of case
Benign tumor	22 (44.9%)
Adenoma	19 (38.8%)
Benign mixed tumor	3 (6.1%)
Malignant tumor	27 (55.1%)
Adenocarcinoma	7 (14.3%)
Solid carcinoma	3 (6.1 %)
Sclerosing carcinoma	5 (10.2%)
Malignant mixed tumor	8 (16.3%)
Malignant myoepithelioma	4 (8.2%)
TOTAL	49

Table 4. Expression of HER-2/neu, EGFR and ALCAM detected by immunohistochemistry

Mammary tumor	Percentage of positive cases (numbers)		
	HER-2/neu	EGFR	ALCAM
Benign tumor	7/22 (31.8%)	6/22 (27.3%)	9/22 (40.9%)
Adenoma	5 (10.2%)	4 (8.2%)	7 (14.2%)
Benign mixed tumor	2 (4.1%)	2 (4.1%)	2 (4.1%)
Malignant tumor	8/27 (29.6%)	6/27 (22.2%)	2/27 (7.4%)
Adenocarcinoma	5 (10.2%)	5 (10.2%)	1 (2.0%)
Solid carcinoma	--	--	--
Sclerosing carcinoma	--	--	--
Malignant mixed tumor	3 (6.1%)	1 (2.0%)	1 (2.0%)
Malignant myoepithelioma	--	--	--

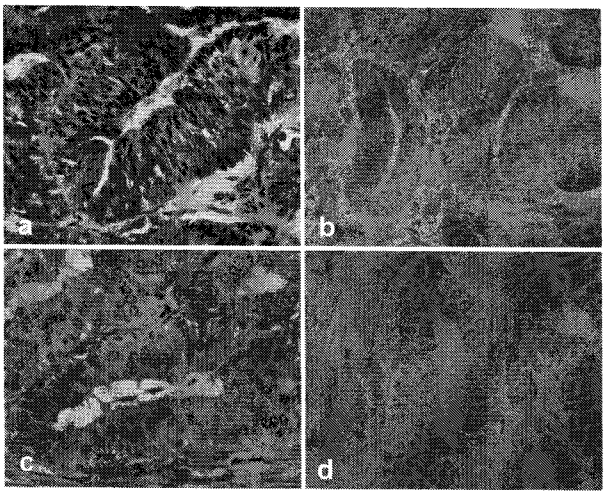

Fig. 1. Mammary gland adenocarcinoma and mammary gland adenoma; dog. a. H&E staining of mammary gland adenocarcinoma (×132); b. Immunohistochemistry of an adenocarcinoma. The neoplastic cell membranes are outlined by a distinct HER-2/neu immunostaining pattern. (ABC method, counterstained with Mayer's hematoxylin, ×132); c. H&E staining of mammary gland adenoma (×132); d. Immunohistochemistry of an adenoma with HER-2/neu expression. There is intense immunoreactivity at the cytoplasmic membrane of neoplastic cells. (ABC method, counterstained with Mayer's hematoxylin, ×132)

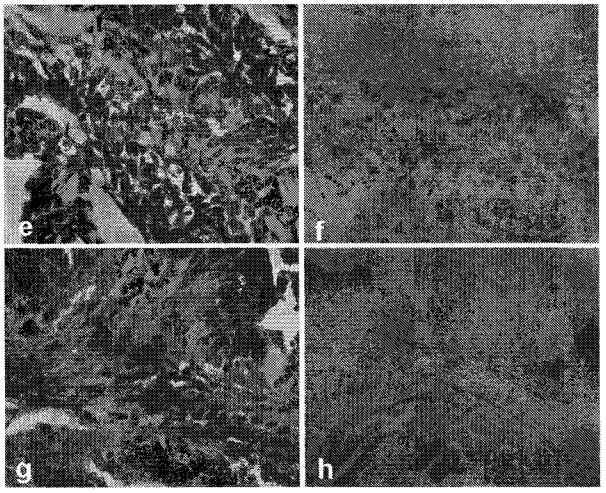

Fig. 2. Mammary gland adenocarcinoma and mammary gland benign tumor; dog. e. H&E staining of mammary gland adenocarcinoma (×132); f. Immunohistochemistry of an adenocarcinoma. There is nuclear of neoplastic cell are by a distinct EGFR immunostining pattern. (ABC method, counterstained with Mayer's hematoxylin, ×132); g. H&E staining of mammary gland benign tumor. (×132); h. Immunohistochemistry of a mammary gland benign tumor. with EGFR expression. There is intense immunoreactivity at the nuclear of neoplastic cells. (ABC method, counterstained with Mayer's hematoxylin, ×132)

whereas only 29.6% (8/27) of the malignant tumors, 5 of which were adenocarcinomas (Fig. 1), were positive.

Expression of the EGFR was detected in 24.5% (12/49) of the mammary tumors. Immunoreactivity was invariably in the cytoplasm and its intensity was variable. Six cases (4 adenoma and 2 benign mixed tumors) of the 22 (27.3%) benign tumors were EGFR positive (Fig. 2). Expression of EGFR was observed in 22.2% (6/27) of the malignant tumors with 5 adenocarcinomas (Fig. 2) and one malignant mixed tumors almost fulfilling the criterion for EGFR positive.

Expression of the ALCAM was detected in 22.4% (11/49) of the mammary tumors. Immunoreactivity was invariably in the cytoplasm and its intensity was variable. 9 (7 adenoma and 2 benign mixed tumor) of 22 (40.9%) of benign tumors showed positive ALCAM immunostaining and 2 (1 malignant mixed tumors and 1 adenocarcinoma) of 27 (7.4%) of malignant tumors expressed ALCAM (Fig. 3).

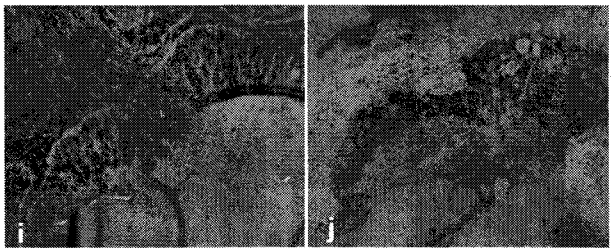

Fig. 3. Mammary gland adenoma; dog. i. H&E staining of mammary gland adenoma (×132); j. Immunohistochemistry of an adenoma. The neoplastic cell membranes are outlined by a distinct ALCAM immunostining pattern. (ABC method, counterstained with Mayer's hematoxylin, ×132)

Discussion

The incidence of canine mammary tumors generally increases with age and such tumors occur rarely in dogs less than 2 years old [2]. The average age was 8.53 years ranging from 2 to 21 years at the time of tumor excision, and the results were comparable to those reported previously in the dogs we studied [7]. Although the lack of sensitivity occurred through high background staining or no cell membrane immunoreactivity, this could be due to differences in fixation, the time lapse from excision to fixation, the actual time that the tissue is in a fixative or in

the immunohistochemical detection system.

Nakopoulou et al [19]. demonstrated that c-erbB-2 membrane staining of breast cancers was due generally to oncogene amplification, although gene amplification does not seem to be the only underlying genetic alteration responsible for c-erbB-2 overexpression. These results suggest that c-erbB-2 expression may be activated during the initial step of canine mammary tumor development, playing a role in malignant tumor development and that the c-erbB-2 expression status may be a useful prognostic indicator in dogs with mammary tumors [1].

Epidermal growth factor (EGF) is a low molecular weight polypeptide that elicits mitogenic and nonmitogenic responses in a variety of cell types. A specific membrane receptor for EGF has been identified and purified. The receptor is a 170 kDa glycoprotein having a single polypeptide chain, In addition to a binding site for EGF, the receptor has intrinsic protein kinase activity that is tyrosine specific and activated by the binding of EGF. The protein kinase characteristics of the EGFR are similar to properties of protein kianse activities possessed by receptors for other hormones that modulate cell growth, such as PDGF and insulin, and certain viral transforming proteins that are also membrane localized. Using immunoistochemistry Moller et al [18] showed EGFR expression in ductal, lobular, and myoepithelial cells, but only occasionally in stroma cells. EGFR is incidence of positivity ranges frequently 30~40% [20] for immunohistochemistry. For the EGFR gene has been a great difference found between the incidence of gene amplification (0~14%) [16] relative to protein expression, in contrast to HER-2/neu in which there is a positive correlation amplification. Amplification of the HER-2/neu gene has been described in approximately 20% of breast tumors (range 8~64%) [11].

Activated leukocyte cell adhesion molecule (ALCAM/CD166) could function as a cell surface sensor for cell density, controlling the transition between local cell proliferation and tissue invasion in melanoma progression. This study is the first to focus on ALCAM expression and its prognostic value in breast carcinoma. Possibilities for this discrepancy may rest with different epithelial/tumor cell content in different tumors and the possible contribution from infiltrating immune cells. Hematopoietic stem cells and activated T lymphocytes express ALCAM [32]. Only two types of cancer have been previously evaluated for ALCAM-melanoma and prostate cancer. With melanoma there was increased staining for ALCAM during the vertical growth phase of the tumor [32]. Immunohistochemical analysis showed that ALCAM up-regulation was found in most low-grade tumors, where as down-regulation occurred preferentially in high-grade tumors, although up-regulation of ALCAM expression was preserved in two Gleason grade 5 tumors [13].

In this study, we showed positive correlations between biology (HER-2/neu, EGFR and ALCAM) and morphology in canine mammary gland tumors. These results indicate that the morphology and biology of canine mammary malignant tumors are closely linked, just as in human breast cancer. ALCAM is a glycoprotein that is involved with cellular adhesion, proliferation, and tumor progression. In mammary carcinoma, decreased ALCAM transcripts in the primary tumors correlate with nodal involvement, higher grade tumors important. The expression of immunoreactive HER-2/neu, EGFR, and ALCAM in canine mammary tumors could be an important factor in canine mammary carcinogenesis.

In conclusion, the immunohistochemical of EGFR, c-erbB-2 and ALCAM protein showed a concordance among the canine mammary gland tumor cases.

Acknowledgment

This research was supported by a grant (code: CBM 31-B3003-01-01-00) from the Center for Biological Modulators of the 21st Century Frontier R&D Program, the Ministry of Science and Technology, Korea.

References

1. Ahern, T. E., R. C. Bird, A. E. Church Bird and L. G. Wolfe. 1996. Expression of the oncogene c-erbB-2 in canine mammary cancers and tumor-derived cell lines. *Am. J. Vet. Res.* **57,** 693-696.
2. Ali, I. U., G. Campbell and R. Lidereau. 1988. Amplification of c-erbB-2 and aggressive human breast tumors? *Science.* **240,** 1795-1798.
3. Anudep, RUNGSIPIPAT., TATEYAMA. Susumu, YAMAGUCHI. Ryoji, UCHIDA. Kazuyuki, MIYOSHI. Noriaki and HAYASHI. Toshiharu. 1999. Immunohistochemical analysis of c-yes and c-erbB-2 oncogene products and p53 tumor suppressor protein in canine mammary tumors. *J. Vet. Med. Sci.* **61(1),** 27-32.
4. Arai, F., O. Ohneda, T. Miyamoto, X. Zhang and T. Suda. 2002. Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation. *J. Exp. Med.* **195,** 1549-1563.

5. Bartkova, J., D. M. Barnes, R. R. Millis and W. J. Gullick. 1990. Immunohistochemical demonstration of c-erbB-2 protein in mammary ductal carcinoma in situ. *Human. Pathology* **21,** 1164-1167.
6. Bowen, M. A., D. D. Patel, X. Li, B. Modrell, A. R. Malacko, W. C. Wang, H. Marquardt, M. Neubauer, J. M. Pesando, U. Francke, B. F. Haynes and A. Aruffo. 1995. Cloning, mapping, and characterization of activated leukocyte-cell adhesion molecule (ALCAM), a CD6 ligand. *J. Exp. Med.* **181,** 2213-2220.
7. Brodey, R. S., M. H. Goldschmidt and J. R. Roszel. 1983. Canine mammary gland neoplasms. *J. Am. Anim. Hosp. Assoc.* **19,** 61-90.
8. Corbel, C., H. G. Bluestein, O. Pourquie, P. Vaigot and N. M. Le Douarin. 1992. An antigen expressed by avian neuronal cells is also expressed by activated T lymphocytes. *Cell. Immunol.* **141,** 99-110.
9. Degen, W., L. Kempen, E. Gijzen, J. van Groningen, Y. van Kooyk, H. Bloemers and G. Swart. 1998. MEMD, a new cell adhesion molecule in metastasizing human melanoma cell lines, is identical to ALCAM (activated leukocyte cell adhesion molecule). *Am. J. Pathol.* **152,** 805-813.
10. Fidler, I. J. and R. S. Brodey. 1967. A necropsy study of canine malignant mammary neoplasm. *J. Am. Vet. Med. Assoc.* **151,** 710-715.
11. Gullick, W. J. 1990. The role of the epidermal growth factor receptor and the c-erbB-2 protein in breast cancer. *Int. J. Cancer.* **5,** 55-66.
12. Harris, C. C. and M. Hallstein. 1993. Clinical implication of the p53 tumor suppressor gene. *New. Engl. J. Med.* **329,** 1318-1327.
13. King, J. A., S. F. Ofori-Acquah, T. Stevens, A. B. Al-mehdi, O. Fodstad and W. G. Jiang. 2004. Activated leukocyte cell adhesion molecule in breast cancer: Prognostic indicator. *Breast Cancer Res.* **6,** R478-R487.
14. Klijn, J. G., P. M. Berns, P. I. Schmitz and J. A. Foekens. 1992. The clinical significance of epidermal growth factor receptor (EGF-R) in human breast cancer: a review of 5232 patients. *Endocr. Rev.* **13,** 3-17.
15. Krupp, M. N., D. T. Connolly and M. D. Lane. 1982. Synthesis, turnover and down-regulation of epidermal growth factor receptors in human A431 epidermal carcinoma cells and skin fibroblasts. *J. Biol. Chem.* **257,** 11489-11496.
16. Lacroix, H., J. D. Iglehart, M. A. Skinner and M. H. Kraus. 1989. Overexpression of erbB-2 or EGF receptor proteins present in early stage mammary carcinoma is detected simultaneously in matched primary tumors and regional metastases. *Oncogene* **4,** 145-151.
17. Miller, W. R., M. O. Ellis, J. R. C. Sainsbury and J. M. Dixon. 1994. Prognostic factors ABC of breast disease. *Br. Med. J.* **309,** 1573-1576.
18. Moller, P., G. Mechtersheimer, M. Kaufmann, G. Moldenhauer, F. Momburg and T. Mattfeldt. 1989. Expression of epidermal growth factor receptor in benign and malignant primary tumours of the breast. *Virchows. Arch. [Pathol. Anat.]* **414,** 157-164.
19. Nakopoulou, L. L., A. Alexiadou, G. E. Theodoropoulos, A. C. H. Lazaris, A. Tzonou and A. Keramopoulos. 1996. Prognostic significance of the co-expression of p53 and c-erbB-2 protein in breast cancer. *J. Pathol.* **179,** 31-38.
20. Nicholson, R. I., R. A. McClelland, J. M. W. Gee, D. L. Manning, P. Cannon, J. F. R. Robertson, I. O. Ellisand R. W. Blamey. 1994. Epidermal growth factor receptor expression in breast cancer: association with responses to endocrine therapy. *Breast Cancer Res. Treat.* **29,** 117-125.
21. Ohneda, O., K. Ohneda, F. Arai, J. Lee, T. Miyamoto, Y. Fukushima, D. Dowbenko, L. A. Lasky and T. Suda. 2001. ALCAM (CD166): its role in hematopoietic and endothelial development. *Blood* **98,** 2134-2142.
22. Pauletti, G., S. Dandekar, L. Ramos, H. Peng, R. Seshadri and D. Slamon. 2000. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemisty. *J. Clin. Oncol.* **18,** 3651-3664.
23. Rachamandra, S., L. Machin, S. Ashley, P. Monaghan and B. A. Gusterson. 1990. Immunohistochemical distribution of c-erbB-2 in in situ carcinoma: a detailed morphological analysis. *J. Path.* **161,** 7-14.
24. Rungsipipat, A., S. Tateyama, R. Yamaguchi, K. Uchida, N. Myoshi and T. Hayashi. 1999. Immunohistochemical analysis of c-yes and c-erbB-2 oncogene products and p53 tumor suppressor protein in canine mammary tumors. *J. Vet. Med. Sci.* **61,** 27-32.
25. Rosen, A. and I. J. Oberman. 1960. Addiction to phenmetrazine hydrochloride and its psychiatric implications. J. Am. Osteopath. Assoc. **59,** 722-726.
26. Sainsbury, J. R., J. R. Farndon, A. L. Harris and G. V. Sherbet. 1985. Epidermal growth factor receptors on human breast cancers. *Br. J. Surg.* **72,** 186-188.
27. Salomon, D. S., R. Brandt, F. Ciardello and N. Normanno. 1995. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.* **19,** 183-232.
28. Schechter, A. L., D. F. Stern, L. Vaidyanathan, S. J. Decker, J. A. Drebin, M. I. Greene and R. A. Weinberg. 1984. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* **312,** 513-516.
29. Shih, C., L. C. Padhy, M. Murray and R. A. Weinberg. 1981. Transforming genes of carcinomas and neuroblastomas introduced introduced into mouse fibroblasts. *Nature* **290,** 261-264.
30. Slamon, D. J., G. M. Clark and S. G. Wong. 1987. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* **235,** 177-182.
31. Thomas, C. J., W. K. Norval and D. H. Ronald. 1997. *Veterinary pathology.* pp. 1190-1200, In: Genital system, 6th eds., (Williams & Wilkins) Lippincott Baltimore, Maryland.
32. Toi, M., T. Tominaga and A. Osaki. 1994. Role of epi-

dermal growth factor receptor expression in primary breast cancer: results of a biochemical study and an immunocytocehmical study. *Breast Cancer Res. Treat.* **29,** 51-58.

33. van Kempen, L. C., J. J. van den Oord, G. N. van Muijen, U. H. Weidle, H. P. Bloeers and G. W. Swart. 2000. Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. *Am. J. Pathol.* **156(3),** 769-774.

초록 : HER-2/neu 단백질이 개 유방암에서의 발현분석

양해걸 · 도선희[1] · 위엔동웨이 · 홍일화 · 기미란 · 박진규 · 구문정
이혜림 · 홍경숙 · 황옥경 · 한정연 · 박호용[2] · 유성은[3] · 정규식*
(경북대학교 수의과대학 병리학교실, [1]건국대학교 수의과대학 임상병리학교실
[2]Insect Biotec Co., Ltd., [3]한국화학연구원)

개에서의 유선 종양진단은 총 49 case 중에서 Human epidermal growth factor receptor (HER-2/neu, c-erbB-2), Epidermal growth factor receptor (EGFR), Activated leukocyte cell adhesion molecule (ALCAM) 등 면역조직화학적염색법을 실시하였다. 우선 49 case를 두 그룹으로 즉: 양성종양그룹 (22 case)과 악성종양그룹 (27 case)으로 구분하였다. 면역조직화학적염색법의 분석결과 HER-2/neu의 발현은 양성종양에서는 31.8% (7/22), 악성종양에서는 29.6% (8/27)의 발현율을 보였고, EGFR의 발현은 양성종양에서는 27.3% (6/22), 악성종양에서는 22.2% (6/27)의 발현율을 보였으며, ALCAM의 발현은 양성종양에서는 40.9% (9/22), 악성종양에서는 7.4% (2/27)의 발현율을 보였다. 결론적으로 개에서의 유선종양진단의 발현율은 사람에서 보고된 것(25%~30%)과 비슷하게 나타났으며 임상진단분야에서 HER-2/neu 항체로 개에서의 유선종양진단에서 유용한 평가수단으로 적용될 수 있으리라 사료된다.