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Citation	Japanese Journal of Veterinary Research, 46(2-3), 151-151
Issue Date	1998-11-30
Doc URL	<a href="http://hdl.handle.net/2115/2702">http://hdl.handle.net/2115/2702</a>
Type	bulletin (article)
File Information	KJ00003408035.pdf



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Expression of the *c-erbB-2* gene and a mouse mammary tumor virus gene-like sequence in canine mammary tumors

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Recent advances in molecular biology have led to creation of a catalog of genetic alterations in human breast tumors. However, genetic alterations in canine mammary tumors have been poorly understood. In the present study on canine mammary tumors, the author first investigated whether malignancy is associated with overexpression of the oncogene *c-erbB-2*, which is a candidate prognostic indicator in human breast cancer, and, second, if a mouse mammary tumor virus (MMTV) gene-like sequence could be identified.

Tissue samples were collected from 27 mammary tumors surgically removed from dogs. These tumors were classified histologically as adenocarcinoma with lymphatic invasion (six tumors), adenocarcinoma with no lymphatic invasion (four), malignant mixed tumors (eleven), benign mixed tumors (three) and adenoma (three).

Expression of *c-erbB-2* was analyzed by RNA dot blot hybridization using a cloned canine *c-erbB-2* gene fragment and the  $\beta$ -actin gene as probes. The expression level of the *c-erbB-2* gene was determined by dividing the signal intensity obtained from *c-erbB-2* hybridization by that obtained from  $\beta$ -actin hybridization. The expression levels of tumor tissues were compared with that of mammary tissue from a disease-free non-lactating dog. Expression of

the *c-erbB-2* gene in the 27 mammary tumors ranged from 0.4 to 1.37 times the expression level in normal reference tissue. There was no significant association between histological classification and the expression level of *c-erbB-2* (Scheffe's test;  $P \geq 0.66$ ). This result suggests that the histological malignancy of canine mammary tumors, unlike human breast tumors, does not correlate with overexpression of *c-erbB-2*.

To detect the MMTV-like gene in canine mammary tumors, nested polymerase chain reaction (PCR) was performed on tumor DNA extracted from paraffin-embedded sections. Primer pairs were designed to amplify a sequence in the 687 bp sequence of the MMTV *env* gene with low homology to endogenous retrovirus or to any other gene previously reported. Amplification of the sequence was observed in 1 of 4 adenocarcinomas, 1 of 6 adenocarcinomas with lymphatic invasion, 2 of 11 malignant mixed tumors and 1 of 3 benign mixed tumors. The sequence was not found in 3 adenomas and 3 normal mammary tissues. RNA dot blot analysis using the labeled cloned 687 bp sequence showed that an MMTV *env* gene-like sequence was expressed in 1 benign mixed tumor that was positive by PCR. These data indicate the possibility that a functional gene homologous to the MMTV *env* gene was present in some canine mammary tumors.