Report

Expression of epidermal growth factor receptor (EGFR) in non-affected and tumorous mammary tissue of female dogs


Department of Clinical Sciences of Companion Animals and Department of Pathology, Faculty of Veterinary Medicine, and Department of Endocrinology, Faculty of Medicine, Utrecht University, and Division of Endocrine Oncology, Dr Daniel Den Hoed Cancer Center, Rotterdam, and Department of Pathology, University Medical Center, Leiden, The Netherlands

Key words: dog, epidermal growth factor receptor, mammary gland, mammary tumour, steroid receptor

Summary

Epidermal growth factor (EGFR), oestrogen (ER), and progestin (PR) receptor concentrations were determined by radioligand binding assay in non-affected mammary tissues (n = 13) and benign (n = 11) and primary/locally recurrent malignant proliferative mammary lesions (n = 45) and metastases (n = 19) in 65 female dogs.

The number of specimens expressing EGFR was not significantly different among these tissues, but EGFR concentration was lower in metastases (P = 0.02) than in benign or primary/locally recurrent malignant lesions not mixed with non-affected mammary tissue. The presence of non-affected mammary tissue in primary cancer specimens was noticed as a factor that may influence results of receptor measurements. No relation was found between the expression of EGFR and that of ER or PR in non-affected or in tumorous mammary tissues.

It was concluded that in the dog mammary gland EGFR expression is not associated with conditions of steroid receptor absence or biological aggressiveness of neoplastic growth.

Introduction

The growth and development of normal mammary tissue is controlled by multiple endocrine factors and by – often locally produced – stimulatory or inhibitory growth factors [1, 2]. Abnormalities in the expression of factors that take part in signalling pathways involved in this control may lead to aberrant cell growth, that is sometimes neoplastic [3, 4]. An important role has been attributed to enhanced expression of the epidermal growth factor receptor (EGFR) in the genesis of certain types of tumours, such as squamous cell carcinomas [5]. Both EGF and transforming growth factor-α (TGF-α), after binding to the EGFR, may influence the growth of several types of cultured cells, including normal and tumorous mammary cells [2, 6, 7]. The proportion of human breast carcinomas reported to express EGFR varies considerably. EGFR-positivity has been noticed to be associated with an adverse prognosis, with absence of expression of oestrogen (ER) or progestin receptors (PR) and with DNA aneuploidy (reviewed in ref. [8]).

Mammary tumorigenesis in the female dog re-
sembles its human counterpart with respect to its dependence upon ovarian steroids (reviewed in ref. [9]). A large proportion of malignant mammary tumours in the dog are simple (epithelial) carcinomas that often develop distant metastases and are lethal, whereas the smaller group of complex carcinomas (composed of epithelial and myoepithelial tumour cells) are less often so [10]. While most histologically non-affected mammary glands and benign proliferative lesions are ER and PR positive, only about half of the malignant tumours express these receptors and metastases do so even less frequently [11]. These findings suggest that many malignant mammary tumours in the dog become steroid-independent.

In this study EGFR concentrations were analysed in histologically non-affected mammary tissues and in benign and malignant mammary proliferative lesions of female dogs and were compared to ER and PR concentrations, in order to investigate the possibility that enhanced expression of EGFR is associated preferentially with loss of steroid receptors and increased biological aggressiveness [12]. In malignant tumours the EGFR results were also compared to DNA ploidy status as determined by flow cytometry.

**Materials and methods**

**Animals**

EGFR analyses were performed in mammary tissues obtained at surgery or autopsy from female dogs (n = 65) of various breeds or mixed breeding. In two-thirds of the cases samples were among tissues examined for the presence of steroid receptors at an earlier date [11], while in the other one-third the samples were newly collected. Histologically non-affected mammary tissues (n = 13) were studied in 9 dogs with benign (n = 6) or malignant proliferative lesions (n = 3) and in 4 dogs without mammary disease. From 5 dogs of this group tumour tissue was also available for EGFR analysis. Histologically benign proliferative lesions (n = 11) were analysed in 11 dogs and malignant tumours (n = 48) in 46. The latter included 38 primary cancers (including 4 in 2 dogs with duplex, bilateral, primary cancers) and 7 locally recurrent cancers presented after previous surgery. In two dogs only regional lymph node metastases were available for receptor studies and in one dog only a distant (abdominal) node metastasis.

In 13 dogs both primary or locally recurrent cancers were analysed, as well as metastases in regional lymph nodes (n = 9 in 7 dogs) or at distant sites (n = 5 in 5 dogs), or both (1 dog). One dog with a benign tumour had a nodal metastasis that at re-examination by histology was found to originate from a concurrent squamous cell carcinoma of the skin. The EGFR value (48 fmol/mg protein) in this sample was excluded from the present analysis.

Clinical staging of dogs with cancer was done on the basis of the WHO TNM classification for tumours in domestic animals [13]. Involvement of regional lymph nodes was confirmed by cytological or histological examination. Exposure to endogenous progesterone (luteal phase) or to exogenous progestins via injection of long-acting compounds to prevent oestrus was recorded as described previously [11].

**Tissues**

At surgery or autopsy, the latter always being completed within 30 minutes following euthanasia, tissues were placed immediately in melting ice. Macroscopically tumourous and normal tissues, well separated from each other, were partly dissected, cleared of fat and necrotic parts, and cut in blocks of about 0.5 cm diameter. Several blocks and the remaining specimen were fixed in 10% formalin for histopathological examination, according to the WHO guidelines [14]. The other blocks were frozen quickly in liquid nitrogen and stored at – 70° C. Steroid receptor analysis was performed within two months. EGFR analysis took place after 1–4 years of storage.

For these analyses mammary tissues were considered to be non-affected only if no proliferative lesions were found by microscopic examination. The relative proportion of epithelial + myoepithelial cells was assessed as a percentage of tissue in micro-