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Feline mammary tumours in comparative oncology

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Domestic animals as spontaneous models for human cancer

Naturally occurring tumours in domestic animals have been recognized as an interesting opportunity for comparative oncology (MacEwen, 1990; Vail & MacEwen, 2000). Cancer is the second most frequent cause of death in humans and the first one in dogs and cats (Jemal et al. 2003). The age-adjusted overall cancer incidence per 100 000 individuals per year is comparable in humans and domestic animals, being approximately 300 in humans, 381 in dogs and 264 in cats (Vail & MacEwen, 2000). When analysing the incidence by site, breast cancer is the most frequent (32%) in women, the first of all neoplasia (52%) occurring in bitches and the third (17%) in queens after lymphohaemopoietic and skin tumours (Hayes et al. 1981; Hayes & Mooney, 1985; MacEwen, 1990; MacEwen & Withrow, 1996; Jemal et al. 2003). Several other aspects contribute to the value of domestic animals as models for human cancers (MacEwen, 1990; Vail & MacEwen, 2000). Tumours occur spontaneously in companion animals that share a similar environment with humans and therefore might be exposed to similar risk factors. The high incidence of some tumour types offers large population samples. The shorter overall lifespan of domestic animals associated with a more rapid progression of cancer allows adequate comparison of response time with humans. Biological, anatomical, histopathological, genetic, and molecular similarities between some animal and human tumours are also well established (Hansen & Khanna, 2004). Finally, testing novel therapies is more ethically acceptable when treating spontaneous diseases in companion animals rather than experimentally induced pathologies in animal models. At the same time, there is an increasing interest of owners towards the use of the most advanced therapeutic tools for companion animals despite the higher economic costs associated with these therapies.

Mainly based on age incidence, risk factors, histopathology, prognostic aspects, metastatic pattern and response to therapy, feline mammary carcinoma (FMC) has been proposed as a good model for human breast cancer (HBC) (Weijer & Hart, 1983; Stolwijk et al. 1989; Hahn et al. 1994). Here we summarize the characteristics that are shared by mammary gland tumours in cats and humans.

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Comparative anatomy of normal mammary gland

The mammary gland of dog and cat is composed of secretory lobules drained by arborized interlobular ducts leading to lactiferous ducts and to lactiferous sinuses at the base of the teat. The secretory lobules are situated in the subcutis and are formed by tubuloacinar glands and intralobular ducts. The tubuloacinar structures and the intralobular ducts are very similar in structure and both lined by a luminal cuboidal epithelial layer and a basal layer of flattened actin-positive myoepithelial cells. Termination of the intralobular ducts is characterized by cellular thickening (''terminal end buds'') that may represent gland remnants or gland precursors in inactive glands. The interlobular ducts and the sinuses are characterized by a double-stratified epithelium of cuboidal to tall columnar cells and scattered peripheral myoepithelial cells, while a squamous stratified epithelium from the teat canal is continuous with the skin. Multiple lactiferous sinuses and canals pass through a single teat in carnivores (Bacha & Bacha, 2000). Similar features are observed in the human breast (Young & Heath, 2000). Mammary tumours are thought to originate from the intralobular ductal terminal end buds giving rise to terminal ductal neoplasia rather than lobular tumours (Wellings, 1980; Russo & Russo, 1987; Van Garderen et al. 1997). However, since the exact origin of tumoural cells remains to be established, the terms ''ductal'', ''ductular'' and ''lobular'' do not feature in the recent classification of mammary tumours in domestic animals (Misdorp et al. 1999). Lobular and ductal breast carcinomas are still described in humans just on the basis of the resemblance of the involved structure (Fletcher, 1995).

Vascularization and lymphatic communication of mammary glands are fundamental to the understanding of the development of metastasis in tumours. In the cat, there are generally four mammary glands per side referred to as

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axillary, thoracic, abdominal and inguinal. Axillary and thoracic mammary glands are supplied by the perforating branches of the internal thoracic, the intercostal, and the lateral thoracic arteries. Abdominal glands receive blood from the cranial superficial epigastric artery while branches of the external pudendal artery supply the inguinal glands. The veins of the feline mammary glands follow closely the arteries, except for some of them, which cross the midline, eventually allowing metastatic dissemination between paired glands. This is a unique feature of the cat mammary gland (Crouch & Lackey, 1969). A lymphatic network connects the anterior glands (axillary and thoracic) of the same side and drains into the ipsilateral axillary lymph node, while the posterior glands (abdominal and inguinal) have lymphatic connections draining into the superficial inguinal lymph node (Hayden & Nielsen, 1971). In the cat, excision of the single axillary/inguinal lymph node is relatively easy and generally performed when a mammary tumour is diagnosed or suspected. A quite different situation is present in the axillary region of humans. A much higher number (70–80) of axillary lymph nodes form a complex network (pectoral, subscapular, and humeral groups drain the central axillary lymph nodes to the apical axillary ones), draining 75% of lymph from each breast, while the rest goes to the parasternal and abdominal nodes or to the other breast, where a controlateral metastatic tumour may develop (Boova et al. 1982).

Epidemiology and risk factors

Mammary neoplasia is the third most common tumour type affecting female cats. Rare cases in male cats have been reported (Hayes et al. 1981). Mean age of development is 10–11 years with an age-relative risk that increases up to 14 years. Malignancy occurs frequently (80–96%) with high mortality and a ratio between malignant and benign neoplasms varying from 9 : 1 to 4 : 1 (Hayes et al. 1981; Misdorp et al. 1991). Human breast cancer (HBC) is the most commonly represented type of tumour in women with increasing incidence in the last decade and a high rate of malignancy. The incidence rises during lifetime and 77% of cases occur between 50 and 70 years with an average age at diagnosis of 64 years, similar to that described for FMC after adjusting for age (Rhodes, 2002). Breast carcinoma in men is rare (1 : 100 male to female breast cancer) (Giordano et al. 2002).

All feline breeds may be affected. Some studies reported that the Siamese breed has twice the risk of developing mammary cancer. In addition, the mean age at time of diagnosis in Siamese female cats seems to be lower than in other breeds and the age-related risk reaches a plateau earlier (9 years of age). All this evidence suggests a genetic predisposition in this feline breed (Hayes et al. 1981; Ito et al. 1996). The existence of hereditary predisposition is well-known in women. Familial breast carcinomas are often associated with mutations at the BRCA1 and BRCA2 genes. Women carrying these mutations are significantly younger at time of diagnosis (Carter, 2001).

A protective effect of early spaying in cats is well documented. Intact females have a significantly higher risk (seven fold according to Dorn et al. 1968) of developing feline mammary cancer (FMC) than early ovariectomized cats (approximately 0·6% relative risk) (Weijer & Hart, 1983). However, the latest age for spaying to be effective remains to be assessed (Hayes et al. 1981). Compared with the USA, in Europe, where spaying is carried out at an older age (5–6 years), if performed at all, a much higher incidence of mammary tumours is observed in cats and in other domestic species. Regular and prolonged administration of progestagens, applied to prevent oestrus in queens particularly in Europe, increases the risk of mammary tumour development, adding further evidence for the role of sex hormones in the pathogenesis of this malignancy (Misdorp, 1991; Misdorp et al. 1991; Hayes et al. 1992). The influence of steroid hormones on the onset of mammary cancer is well-known also in humans. Young age (<11 years) at menarche increases the risk by up to 20%, as does late menopause, and postmenopausal hormone therapy may also slightly increase the risk in women (Gail et al. 1989; Mahavani & Sood, 2001; Nelson et al. 2002). Diet-associated factors (i.e., fat and obesity) presumably responsible for higher oestrogen levels have been associated with increased risk of breast tumours, while it is markedly decreased (up to 75%) by oophorectomy (Hamajima et al. 2002). No association between parity and mammary tumour risk has been found in cats while early full term pregnancy decreases the risk in women compared with a nulliparous or a late pregnancy history (Weijer & Hart, 1983; Gail et al. 1989). Several environmental factors may be considered risk factors in the development of breast cancer in humans as documented by geographic variation of incidence and for radiation and tobacco exposure (Hamajima et al. 2002). Studies of environmental influence on cats sharing similar habitats with women might be useful in detailing these and others factors and the relative magnitude of risk.

Clinical features, diagnostic procedures and therapy

Feline mammary gland tumours occur either as single or multiple nodules, discrete and palpable or attached to the underlying tissue. Frequently, nodules show ulceration mainly in association with extensive tumoural necrosis. All mammary glands can be affected, but some authors suggest the posterior ones are more frequently involved (Hahn et al. 1994). Multiple mammary nodules in cats are quite frequent. In general, they are located in adjacent ipsilateral glands and are often considered to be caused by lymphogenous spread of a single primary tumour. Contralateral concomitant neoplastic nodules are observed less frequently and may be associated with haematogenous involvement.

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Multiple identical neoplastic lesions in non adjacent glands and carcinoma of different histological type in adjacent or non adjacent glands may also be found (Weijer & Hart, 1983). It is a matter of discussion whether they still have to be considered as metastasis of a single initial tumour or if they represent simultaneous primary tumours (Hahn et al. 1994). Generally at time of diagnosis the tumour is already in an advanced stage owing to both its own rapid progression and to delay in detection and in presentation to veterinarians (Weijer & Hart, 1983). The interval between diagnosis and first operation is usually 5–7 months. The average time between FMC detection and death is reported to be 12·3 months (<6–13 months as interval) (Weijer et al. 1972; Hayes et al. 1981; Hayes & Mooney, 1985; Hahn et al. 1994).

In women with breast tumour, palpation of a discrete solid breast mass (usually >2 cm in diameter) is the most common finding. Pain, nipple retraction and discharge, skin changes, such as fixation, dimpling, oedema, redness, and in advanced stages, thickening and ulceration are also features of breast neoplasia (Barton et al. 1999). The upper outer quadrant is the most commonly affected site (50%).

FMC are highly infiltrative and metastasizing tumours. Major sites of metastasis are regional lymph nodes, lungs, pleura, and liver (Weijer et al. 1972; Stolwijk et al. 1989). Some authors report 93% of cats with metastasis at necropsy (82·8% lymph nodes, 83·6% lungs, 42·2% pleura and 23·6% liver; Hahn et al. 1994). Paraneoplastic syndromes are uncommon. Respiratory signs develop in cats with extensive lung involvement and pleural carcinomatosis. Metastatic pattern of breast cancer in women is similar to that described in cats, regional lymph nodes and lungs being the major sites involved (Hahn et al. 1994).

A special type of highly aggressive and infiltrative carcinoma (inflammatory breast/mammary carcinoma), traditionally reported in women and bitches and clinically characterized by severe and diffuse skin reddening, erythema, oedema, firmness, and pain due to embolic dissemination in superficial dermal lymphatics even without a discrete mass, has been recently recognized also in cats, and it might therefore represent a new model to study the disease in humans (Perez-Alenza et al. 2004).

Generally, clinical examination and palpation of mammary nodules or masses is highly suggestive of mammary tumours. In cats, severe dysplastic and inflammatory lesions of mammary glands may also present as diffuse or lobulated masses and dysplastic/neoplastic growth from other tissues may cause development of masses within the mammary region (epidermal cysts, follicular tumours, sebaceous/sweat gland tumours, round cells tumours, i.e., mast cell tumours, fibrosarcoma of the dermis). Cytology specimens from the lesions may indicate an atypical epithelial glandular overgrowth eventually allowing differentiation with inflammation, but distinguishing between benign and malignant mammary gland tumours may be difficult. Histology is normally needed to confirm the diagnosis and to classify the lesion. In humans, imaging systems are the major diagnostic method for breast cancer and allow identification of early clinically insignificant node-negative tumours at curable stage without the delay evidenced in the feline species. Fine-needle aspiration and biopsies are performed to investigate the nature of every imaging detected or palpable breast mass (Barton et al. 1999). TNM staging system is currently used both in cats and women (Owen, 1980). It relies on the size of primary tumour, the involvement of lymph nodes and the development of distant visceral metastasis. In humans the TNM system is applied within the Union Internationale Contre Cancer (UICC) (Sobin & Wittekind, 1997). A second American Joint Committee (AJCC) on Cancer Staging system is widely used. It relies on a different nomenclature, but it is based on identical criteria (Greene et al. 2002).

Surgical excision of breast/mammary tumours is generally the treatment of choice. In cats it may include nodulectomy, resection of the affected mammary gland with or without removal of draining nodes, and total monolateral or bilateral mastectomy. The effect of ovariohysterectomy (see below, ''prognostic factors'') at time of mammary excision has been long discussed. It is now generally maintained that there is no influence on either the development of new benign tumours or the progression of carcinoma (Misdorp et al. 1991). In HBC the decision of aggressive surgical approach *v.* local lumpectomy is generally based on extension and axillary involvement of the lesion. Radiation and multidrug chemotherapy is generally added to surgical excision of breast neoplastic nodules to control dissemination of micrometastasis (Goldhirsch et al. 2001). In cats, although carcinoma at time of diagnosis is generally too extensive and infiltrative for chemotherapy to be significantly helpful, some antineoplastic drugs used have had some effect (5 fluorouracil, doxorubicin, cyclophosphamide, methotrexate, prednisone, vincristina; Stolwijk et al. 1989). In particular, associations of doxorubicin and cyclophosphamide may induce response in 50% of cats with unresectable or metastatic neoplasia, even if they have to be carefully applied since side effects such as nephrotoxicity, myelosuppresion and anorexia have been described (MacEwen, 1990; Vail & MacEwen, 2000). Endocrine therapy (i.e., tamoxifen) is widely used to treat oestrogen-receptor positive breast tumours in humans. Little is known about the effect of anti-oestrogen therapy in domestic animals. Relatively few studies have been devoted to this issue. No evidence of positive effects was found in dogs and no significant effect of these treatments was observed in cats (Cappelletti et al. 1988; Morris et al. 1993).

Histology

Histogenetic, descriptive morphology and prognostic aspects might be used to classify mammary gland neoplasia. However, the specific cell type of origin of mammary tumours is still uncertain, as well as the role

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of the myoepithelial component. The histotype appears to be prognostic only in the canine species. Feline mammary tumours are mainly classified on the basis of morphological criteria (Misdorp et al. 1999; Meuten, 2002). FMC are essentially divided into *in situ* malignancies (non-infiltrating-*in situ*-carcinomas) and different types of infiltrative carcinomas. In women mammary carcinomas are similarly classified as non-infiltrative neoplasms (15–30% of carcinomas) or invasive carcinomas, and different subtypes are identified by morphology. As already mentioned, a distinction in ductal and lobular tumours (both *in situ* and infiltrating) is used in HBC without implying a site or cell of origin (Fletcher, 1995). *In situ* carcinoma may be difficult to distinguish from atypical hyperplasia both in cats and in women. Non-infiltrative malignancies may present different patterns that are observed in both species: cribriform, solid and comedo-like, and papillary (mainly in women). Although lack of invasion of the basal membrane is not always easy to determine on H&E sections, cats generally show clearly and highly infiltrative tumours at time of diagnosis. Likewise, the ''no special type'' infiltrative carcinoma is the most frequently recognized type of breast tumour (70–80%) in women (Fletcher, 1995). Infiltrative carcinoma may be subdivided into papillary, tubular/cribriform (both very frequent in cats) and solid. Special types have been classified as mucinous and squamous cells carcinomas both in cats and in women, while a medullary invasive carcinoma is described only in humans. The grading system of mammary gland malignancies into well-differentiated (WDC), moderately differentiated (MDC) and poorly differentiated (PDC) carcinomas is based on identical criteria in cats and humans. Specifically, evaluation of degree of tubules formation, nuclear and cellular pleomorphism and mitotic count is generally used as a semiquantitative method in cats as in humans (Scarff-Bloom-Richardson system; Elston & Ellis 1991; Castagnaro et al. 1998a). Briefly, the three parameters are scored from 1 to 3 and then added allowing classification as follows: grade I (WDC), 3–5 points; grade II (MDC), 6–7 points; grade III (PDC), 8–9 points. Interestingly, a similar pattern of distribution of carcinomas has been evidenced, when comparing HBC and FMC, that showed 20% WDC, 42% MDC, 32% PDC and 16% WDC, 50% MDC, 27% PDC, respectively (Castagnaro et al. 1998a). Among benign lesions (both hyperplastic and neoplastic) some morphological similarities are shared by humans and cats. In women, however, these lesions tend to evolve towards cancer as reflected by their classification (nonproliferative–fibrocystic–breast changes; proliferative breast changes without atipia; proliferative breast changes with atipia; Fletcher, 1995). Fibroadenomas are the most frequent benign lesions in both species and often are thought to be associated with steroid hormone excess (feline fibroadenomatous change) (Hayden et al. 1981; Fletcher, 1995; Misdorp et al. 1999; Martin de las Mulas et al. 2000a; Meuten, 2002; Kumar et al. 2004). Myoepithelial cell proliferation may be present in association with epithelial cells (complex tumour), generally in benign neoplasia. Complex malignancies may be infrequently observed both in cats and women (Fletcher, 1995; Meuten, 2002).

Prognostic factors

The most important prognostic factors of mammary gland neoplasia common to women and cats are tumour size and lymph node metastasis, which are also significantly correlated one to each other (MacEwen et al. 1984; Ito et al. 1996; Kumar et al. 2004). Other relevant aspects shared by the two species are histology, grading, therapy and expression of proliferation markers. Prognosis is generally assessed as the 1-year post-surgical rate of survival/remission in cats, which is comparable to the 10-year post-surgical survival/remission rate generally used in humans (MacEwen et al. 1984). Disease-free interval and post-surgical remission rate at a fixed interval are considered better prognostic indicators, at least in cats, since they take into account that survival might be influenced by other concomitant diseases and the ''pure'' tumoural effect might be difficult to assess.

It is well established that tumour size is the most important prognostic parameter in cats, significantly affecting both disease-free interval and survival time. There are several reports showing that subjects with larger lesions have a worse prognosis than those with smaller tumours. Particularly, both tumour volume and tumour diameter have been evaluated. Lesions between 1 cm^3 and 8 cm^3 more frequently show absence of recurrence for a longer period and longer survival time than those with a volume between 9 $cm^3$and 27 cm^3. Significant prognostic differences have been found also between tumours included in this latter range and lesions with volume >27 cm^3 (MacEwen et al. 1984; Hahn et al. 1994; Ito et al. 1996). According to Weijer & Hart (1983) tumour diameter is a more reliable prognostic indicator than tumour volume, and the relationship between diameter and survival is preserved even after correction for necrosis. Lesions with >3 cm diameter show shorter survival time (4–6 months) than those with a diameter between 2 cm and 3 cm (20 months) or <2 cm (>3 years survival). The 1-year post-surgical survival rate and the 1-year post-surgical disease-free rate are also influenced by tumour diameter, being significantly higher for lesions <3 cm and dramatically dropping for lesions close to 6 cm (Weijer et al. 1972, Weijer & Hart, 1983; Hayes & Mooney, 1985; Castagnaro et al. 1998a). In women, tumour size is second only to lymph node involvement as a prognostic indicator. Some reports describe that 52% of women with tumours <2·5 cm survive 10 years while only 25% of women with tumours >2·5 cm have a survival time of 10 years (MacEwen et al. 1984). Tumour size is significantly related to probability of developing breast tumour metastasis.

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The percentage of lesions that develop metastasis is 20% for those <3 cm in diameter and 40–50% for lesions of 3–6 cm in diameter. It increases dramatically for lesions >6 cm, similarly to that seen in cats (Kumar et al. 2004).

Axillary lymph node status is the most important independent prognostic factor in women. The 10-year disease-free survival rate is close to 70–80% for node-negative tumours while it decreases to 10–40% in node-positive ones (depending on the number of nodes affected) (Kumar et al. 2004). Particularly, macrometastasis (>0·2 cm) are of well established prognostic importance, while the prognostic value of micrometastasis, detected by immunohistochemistry or reverse-trascriptase PCR, remains to be quantified (Lara et al. 2003). Many veterinary studies describe lymph node involvement (positive regional superficial inguinal lymph node at histology) as one of the most important prognostic factors for feline mammary tumours (Weijer et al. 1972, Weijer & Hart, 1983; Hayes & Mooney, 1985; MacEwen et al. 1984; Hahn et al. 1994; Ito et al. 1996; Castagnaro et al. 1998a). These major prognostic factors together with metastasis at distant sites are used to stage mammary neoplasia and to give a significant prognostic indication (TNM staging in cats and AJCC on Cancer Staging in humans; Owen, 1980; Greene et al. 2002).

Histological subtypes of breast cancer have been described as prognostic in humans. Although the subtype morphology has not been found to be related to prognosis in cats, the major distinction between *in situ* carcinoma and infiltrative malignancies is of common prognostic relevance in both species on the basis of the obvious capability of invasive carcinoma to metastasize (Weijer & Hart, 1983; Misdorp et al. 1999).

Grading of FMC is based on degree of tubule formation, nuclear and cellular pleomorphism, and mitotic count as summarized by Castagnaro et al. (1998a). Particularly, grade I and grade III lesions appear to have a good predictive value based on the 1-year post-surgical survival rate reported as 100% for grade I tumours, 50% for grade II, and 0% for grade III. Similar prognostic influences have been described for breast cancer grading in women with significantly higher survival rate for grade I tumours than for grade II and grade III (85%, 60% and 15% 10-year survival rate, respectively; Simpson & Page, 1992).

The type of therapeutic approach may obviously affect prognosis of feline mammary neoplasia and human breast cancers. Compared with nodulectomy, complete mastectomy shows a significantly longer disease-free interval in cats (MacEwen et al. 1984; Ito et al. 1996). Animals responding to cyclophosphamide-doxorubicin treatments present a longer survival time (283 d) than non responders (57 d) (Stolwijk et al. 1989).

Proliferative rate is also a prognostic factor in both HBC and FMC. It may be assessed by flow cytometry (S-phase fraction), by mitotic index count at histology and by immunohistochemical detection of specific cellular proteins. Some of these latter proliferation markers (AgNOR count, PCNA, and Ki-67 index) have been studied in FMC and, particularly, AgNOR count has shown an important correlation with survival rate. In humans these markers typically correlate in many tumours with early relapse, metastatic potential and survival rate. A statistically significant variation in the AgNOR count has been found in FMC with respect to the 1-year post-surgical survival, being the count significantly higher in those lesions carried by subjects who died within 1 year after surgery (Castagnaro et al. 1998b). Controversial results have been described for the Ki-67 index. Some authors suggest a lack of prognostic significance of Ki-67 index (Millanta et al. 2002). In our hands, it appears to be significantly correlated with a more aggressive behaviour of FMC (with a cut-off point of 25·2) being higher in tumours that belonged to cats that died before 1 year post surgery (Castagnaro et al. 1998c). Presumably, standardization of methods will be necessary to compare results and to specify the effective role of this marker as an indicator for the biological behaviour of FMC in cats. Both in HBC and in FMC a significant positive correlation between AgNOR and Ki-67 counts has been described, that might therefore pose a significant prognostic role of Ki-67 in these tumours (Bostock et al. 1992, Castagnaro et al. 1998b). PCNA detection in FMC showed a significant difference between malignant and benign lesions. In addition, some authors reported that the PCNA value is correlated with mitotic index in mammary carcinomas when typical and atypical mitotic figures are added together (Preziosi et al. 1995).

Molecular findings and hormonal status

Changes in the expression of many genes at the mRNA and protein level have been reported in mammary carcinomas. Previously recognized morphological subtypes of breast tumours may be identified on the basis of protein expression and gene mutations. Important genes commonly targeted in breast cancer and recently studied also in feline mammary tumours are the human epidermal growth factor receptor-2 (HER2, c-erbB-2, HER2/neu) and the RON gene (tyrosine kinase receptor gene) (De Maria et al. 2002; De Maria et al. 2003). HER2 gene amplification/protein over-expression has been detected in 20–30% of HBC. HER2-positive carcinomas tend to be poorly differentiated and this gene status is an independent predictive factor of a worse prognosis in node-positive patients (Hayes & Thor, 2002). Recently the HER2 gene transcript has been partially sequenced in cats and has revealed a 90–95% homology with the canine and the human sequence respectively. HER2 protein has been found to be over-expressed in 30% of FMC, similarly to that described in humans (De Maria et al. 2003). The RON protein is a member of the MET tyrosine kinase receptor family involved in the activation of the signalling cascade responsible for invasive properties of neoplastic cells. The MET gene family encodes the human MET and RON

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receptors and their mouse homologues (MET and *stk*, respectively). The human RON gene is over-expressed in HBC (Tuck et al. 1996). Recently the feline *stk* gene has been sequenced and found highly homologous to the RON human gene. Feline *stk* gene expression in FMC has been studied revealing a pattern highly similar to human breast tumours, being over-expressed more than 20-fold in 20% of the feline cases examined (De Maria et al. 2002).

Some recent studies on other molecules such as p53, cyclin A, metallothioneins, VEGF, chemokine receptor CXCR4, E-cadherin and BCAR1/p130C confirmed that FMC are somehow similar to HBC. Mutation in the p53 tumour suppressor gene is one of the most common findings in human cancer. Some malignancies of domestic animals also show p53 mutation and over-expression. Some HBC subtypes (particularly basal-like carcinomas) show an increased expression of p53. Similarly, 17·3% of FMC were found to express this protein (wild type and mutant form were not differentiated) while negative results were detected for benign lesions (Murakami et al. 2000a; Nasir et al. 2000). Regulatory proteins of the cyclin family play an important role in the regulation of cell cycle. Both cyclin A and cyclin D1 have been found over-expressed in several human tumours. Mainly cyclin D1 seems to correlate with human breast tumourigenesis and aberrant cyclin A has been evidenced in breast tumours. FMC showed over-expression of cyclin A by IHC (Murakami et al. 2000a,b). Metallothioneins (MT) are low molecular weight proteins characterized by selective affinity for heavy metals (mainly Zn and Cu) and their role in carcinogenesis has been studied in several human tumour types. MT expression has been associated with poor prognosis in human ductal carcinoma of the breast. In cats immunoreactivity to MT was detected only in FMC (30%) while it was not revealed in benign lesions, opposite to that evidenced in canine mammary tumours, where expression was higher in adenomas (Dincer et al. 2001). In HBC, VEGF has been described as of prognostic relevance, being correlated with early relapse and shorter survival. Recent studies reveal a high expression of VEGF in poorly differentiated FMC and significant correlation with histology type, grading and poor prognosis was detected (Millanta et al. 2002). Metastasis development of HBC has been recently related to the use of chemokine receptor (CXCR4) pathway by neoplastic cells. Interestingly, higher expression of CXCR4 has been found in metastatic foci of FMC than in primary neoplastic cells and generally this receptor is more expressed in FMC when compared with normal mammary tissue. In addition, P130CAS adhesion protein, known to be associated with cell migration, is increased in invasive FMC compared with non-invasive and benign lesions (Tanabe et al. 2002; Dias Pereira et al. 2003; Oonuma et al. 2003). Cadherins are generally less expressed in several human tumours including those from breast. Reduction or absence of E-cadherin expression and abnormalities in the pattern of immunostaining were evidenced in a subgroup of FMC while a strong immunoreactivity was detected in normal mammary gland tissues (Scibelli et al. 2003).

Female steroid hormones are associated with mammary tumour development both in domestic animals and in humans. In mammary gland, both normal and neoplastic tissues show concomitant expression of different hormone receptors (Martin et al. 1984). Oestrogens can directly stimulate growth mainly of both interlobular and intralobular ducts and induce progesterone receptors (PR) expression (Hahn et al. 1994). Progesterone stimulates development of the tubular-alveolar units and regulates growth hormone expression (Lantinga-van Leewen et al. 2000). Oestrogen receptors (ER) expression in HBC is routinely evaluated by immunological techniques. ER+ tumours show a better prognosis and 80% of cases tend to respond to hormonal treatments mainly when ER+/PR+ (Valavaara et al. 1990). ER+ breast carcinomas (70–80%) are usually well differentiated and are thought to arise from a ER+ luminal cell. They generally do not express proliferation markers (Palmieri et al. 2002). ER− carcinomas are poorly differentiated and more aggressive and generally do not respond to tamoxifen therapy (Johnston et al. 1995). An interesting situation is presented in cats that tend to have ER− highly aggressive mammary tumours (80%) and therefore might represent a good model for late-stage HBC (Martin de las Mulas et al. 2000b). ER status and associated prognostic considerations are mainly based on ERalpha. Splicing isoforms of this receptor have been described both in humans and in cats (Bargelloni et al. 2002; Hirata et al. 2003), while absent in all experimental rodents, and they show a specific trend when analysed in normal or neoplastic mammary tissue with some similarities between the two species, even if their precise role remains to be defined. These findings related to ER isoforms therefore add value to the feline species as a potential model for human breast neoplasia. In 1996, a second oestrogen receptor (ERbeta) was discovered (Mosselman et al. 1996). Its influence in mammary tumour development is highly controversial: both a protective role and a negative prognostic influence of ERbeta are supported in the literature (Speirs et al. 1999, Fuqua et al. 2003; Nakopoulou et al. 2004). ERbeta has been recently sequenced and studied in feline mammary tumours and preliminary evidence might suggest a negative prognostic role (V Zappulli, unpublished observations).

Progesterone receptor expression analysis in FMC led to controversial results. Recently, a significant correlation of PR positivity with absence of ovariectomy has been reported and generally a decrease in malignant lesions has been observed (65–67% PR+ benign mammary tumour *v.* 37–38% PR+ FMC) by IHC (Martin de las Mulas et al. 2002). PR in breast/mammary tumours of humans and cats is frequently linked with ER expression and considered of positive prognostic value (Johnston et al. 1984; Rutteman et al. 1991). The phenotype ER+/PR+ is the most frequently found in both species but ER+/PR− and a relevant number of ER−/PR+ cases have been described in FMC

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(Martin de las Mulas et al. 2002). The latter cases might suggest the presence of other proliferative regulators apart from oestrogens.

The Cinderella of the mammary gland: the myoepithelial cells

The role of myoepithelial cells in mammary gland tumour development is highly controversial as is their implication in the bone and cartilage metaplasia that may accompany some of these neoplasias (mainly in dogs). Myoepithelial cells represent a natural border separating proliferating epithelial cells from basement membrane and underlying stroma and produce *in vitro* extracellular matrix containing sequestered proteinase inhibitors that reduce tumour cell invasion down to 40% (Sternlicht et al. 1997). Adenomyoepithelioma and adenoid cystic carcinoma of mammary gland have a better long-term follow-up as compared with other ''simple'' epithelial cancer composed exclusively of luminal epithelial cells (Fletcher, 1995). A protective effect of these actin-positive cells in rare feline mammary lesions presenting a myoepithelial component is unclear. Grade II FMC with high actin immunoreactivity showed higher survival rate (Castagnaro et al. 1998a).

In conclusion, on the basis of the features we have summarized here, feline mammary tumours may be considered a good model for their human counterpart, mainly for those late-stage oestrogen-negative invasive breast cancers.

References

Bacha WJ & Bacha LM Jr 2000 *Color Atlas of Veterinary Histology Second Edition*. New York: Lippincott Williams & Wilkins.

Bargelloni L, Zappulli V, Petterino C, Patarnello T, Castagnaro M 2002 Isolation and sequencing of estrogen-receptor alpha cDNA in the cat: potentials for comparative oncology and molecular diagnostics. Proceedings of the 20th ESVP Meeting, Grugliasco (TO) Italy, 18–21Sept

Barton MB, Elmore JG, Fletcher SW 1999 Breast symptoms among women enrolled in a health maintenance organization: frequency, evaluation and outcome. *Annals of Internal Medicine* **130** 651–657

Boova RS, Bonanni R, Rosato FE 1982 Patterns of axillary nodal involvement in breast cancer: predictability of level one dissection. *Annals of Surgery* **196** 642–644

Bostock DE, Moriarty J & Crocker J 1992 Correlation between histologic diagnosis, mean nucleolar organiser region count and prognosis in canine mammary tumours. *Veterinary Pathology* **29** 381–385

Cappelletti V, Granata G, Miodini P, Coradini D 1988 Modulation of receptor levels in canine breast tumours by administration of tamoxifen and etretinate either alone or in combination *Anticancer Research* **8** 1297–1301

Carter RF 2001 BRCA1, BRCA2 and breast cancer: a concise clinical review. *Clinical and Investigative Medicine* **24** 147–157

Castagnaro M, Casalone C, Bozzetta E, De Maria R, Biolatti B, Caramelli M 1998a Tumour grading and the one-year post-surgical prognosis in feline mammary carcinomas. *Journal of Comparative Pathology* **119** 263–275

Castagnaro M, Casalone C, Ru G, Nervi GC, Bozzetta E, Caramelli M 1998b Argyrophilic nucleolar organizer regions (AgNOR) count as indicator of post-surgical prognosis in feline mammary carcinomas. *Research in Veterinary Science* **64** 97–100.

Castagnaro M, De Maria R, Bozzetta E, Ru G, Casalone C, Biolatti B, Caramelli M 1998c Ki-67 index as indicator of the post-surgical prognosis in feline mammary carcinomas. *Research in Veterinary Science* **65** 223–226.

Crouch JE & Lackey MB 1969 The mammary gland – Its structure, relationships and blood supply. In: *Text-Atlas of Cat Anatomy*, p. 183 (Eds Crouch JE & MB Lackey). Philadelphia: Lea & Febinger

De Maria R, Iussich S, Olivero M, Di Renzo MF, Biolatti B 2003 Her2/neu oncogene expression in feline mammary carcinomas. Proceedings of the 21st ESVP Meeting, Dublin, Ireland, 13 Sept

De Maria R, Maggiore P, Biolatti B, Prat M, Ciomoglio PM, Castagnaro M & Di Renzo MF 2002 Feline STK gene expression in mammary carcinomas. *Oncogene* **21** 1785–1790

Dias Pereira P & Gartner F 2003 Expression of E-cadherin in normal, hyperplastic and neoplastic feline mammary tissue. *Veterinary Record* **153** 297–302

Dincer Z, Jasani B, Haywood S, Mullins JE, Fuentealba JC 2001 Metallothionein expression in canine and feline mammary and melanotic tumours. *Journal of Comparative Pathology* **125** 130–136

Dorn CR, Taylor DON, Schneider R, Hibbard HH & Klauber MR 1968 Survey of animal neoplasms in Alameda and Contra Costas counties, California. II. Cancer morbidity in dogs and cats from Alameda County. *Journal of the National Cancer Institute* **40** 307–318

Elston CW & Ellis IO 1991 Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* **19** 403–410

Fletcher CDM 1995 *Diagnostic Histopathology of Tumors Volume 1*. New York: Churchill Livingstone

Fowler EH, Wilson GP, Koestern AA 1974 Biologic behaviour of canine mammary neoplasm based on a histogenic classification. *Veterinary Pathology* **11** 212–229

Fuqua SAW, Schiff R, Parra I, Moore JT, Mohsin SK, Osborne CK, Clark GM & Allred DC 2003 Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. *Cancer Research* **63** 2434–2439

Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, Mulvihill JJ 1989 Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *Journal of the National Cancer Institute* **81** 1879–1886

Giordano SH, Buzdar AU, Hortobagyi GN 2002 Breast cancer in men. *Annals of Internal Medicine* **137** 678–687

Goldhirsch A, Glick JH, Gelber RD, Coates AS & Senn H-J 2001 Meeting highlights: International Consensus Panel on the Treatment of Primary Breast Cancer. Seventh International Conference on Adjuvant Therapy of Primary Breast Cancer. *Journal of Clinical Oncology* **19** 3817–3827

Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M 2002 *AJCC Cancer Staging Manual, 6th edition*. New York: Sprinter

Hahn KA, Bravo L & Avenell JS 1994 Feline breast carcinoma as a pathologic and therapeutic model for human breast cancer. *In Vivo* **8** 825–828

Hamajima N et al. 2002 Alcohol, tobacco and breast cancer: collaborative reanalysis of individual data form 53 epidemiological studies, including 58 515 women with breast cancer and 95 067 women without the disease. *British Journal of Cancer* **87** 1234–1245

Hansen K & Khanna C 2004 Spontaneous and genetically engineered animal models: use in preclinical cancer drug development. *Europena Journal of Cancer* **40** 858–880

Hayden DW & Nielsen SW 1971 Feline mammary tumours. *Journal of Small Animal Practice* **12** 687–698

Hayden DW, Johnstone SD & Kiang DT et al. 1981 Feline mammary hypertrophy/fibroadenoma complex: clinical and hormonal aspects. *American Journal of Veterinary Research* **42** 1699–1703

https://doi.org/10.1017/S0022029905001263 Published online by Cambridge University Press

Hayes AH & Mooney BA 1985 Feline Mammary Tumors. Symposium on Clinical Veterinary Oncology. *Veterinary Clinics of North America: Small Animal Practice* **15** 513–520

Hayes DF & Thor AD 2002 C-erbB-2 in breast cancer: development of a clinically useful marker. *Seminars in Oncology* **29** 231–245

Hayes HM, Milne KL & Mandell CP 1981 Epidemiological features of feline mammary carcinoma. *Veterinary Record* **108** 476–479

Hayes, Misdorp W, Romijn A & Hart AA 1992 The significance of ovariectomy and progestagens in the development of mammary carcinoma in cat. *Tijdschr Diergeneeskd* **117** 2–4

Hirata S, Shoda T, Kato J, Hoshi K 2003 Isoform/variant mRNAs for sex steroid hormone receptors in humans. *Trends in Endocrinology and Metabolism* **14** 124–129

Ito T, Kadosawa T, Mochizuki M, Matsunaga S, Nishimura R & Sasaki N 1996 Prognosis of malignant mammary tumours in 53 cats. *Journal of Veterinary Medical Science* **58** 723–726

Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ 2003 Cancer statistics, 2003. *Cancer Journal for Clinicians* **53** 5–26

Johnston SD, Hayden DW, Kiang DT, Handschein B & Johnson KH 1984 Progesterone receptors in feline mammary adenocarcinoma. *American Journal of Veterinary Research* **45** 379–382

Johnston SRD, Saccani-Jotti G, Smith IE, Salter J et al. 1995 Changes in estrogen receptor. Progesterone receptor, and pS2 expression in tamoxifen-resistant human breast cancer. *Cancer Research* **55** 3331–3338

Kumar V, Abbas AK, Fausto N 2004 *Robbins and Cotran Pathologic Basis of Disease. 7th Edn* Elsevier Saunders

Kurtzman ID & Gilbertson SR 1996 Prognostic factors in canine mammary tumours. *Seminars of Veterinary Medicine and Surgery* **1** 25–32

Lantinga-van Leewen IS, van Gardeen E, Rutteman GR & Mol JA 2000 Cloning and cellular localization of the canine progesterone receptor: co-localization with growth hormone in the mammary gland. *Journal of Steroid Biochemistry and Molecular Biology* **75** 219–228

Lara JF, Young SM, Velilla RE, Santoro EJ & Templeton SF 2003 The relevance of occult axillary micrometastasis in ductal carcinoma in situ: a clinicopathologic study with long-term follow-up. *Cancer* **98** 2105–2113

MacEwen EG, Hayes AA, Harvey HJ, Patnaik AK, Mooney S & Passe S 1984 Prognostic factors for feline mammary tumors. *Journal of the American Veterinary Medical Association* **2** 201–204

MacEwen EG 1990 Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. *Cancer and Metastasis Review* **9** 125–136

MacEwen EG & Withrow SJ 1996 Tumours of the mammary gland. In: *Small Animal Clinical Oncology,* pp. 576–602 (Eds SJ Withrow & EG MacEwen). Philadelphia PA, USA: WB Saunders Company

Mahavni V & Sood Anil K 2001 Hormones replacement therapy and cancer risk. *Current Opinion in Oncology* **13** 384–389

Martin de las Mulas JM, van Niel M, Millan Y, Blankenstein MA, van Mil F & Misdorp W 2002b Immunohistochemical analysis of estrogen receptors in feline mammary gland benign and malignant lesions: comparison with biochemical assay. *Domestic Animal Endocrinology* **18** 111–125

Martin de las Mulas M, Millan Y, Bautista MJ, Perez J & Carrasco L 2000 Estrogen and progesterone receptors in feline fibroadenomatous change: an immunohistochemical study. *Research in Veterinary Science* **68** 15–21

Martin de las Mulas M, van Niel M, Millan Y, Ordas J, Blankenstein MA, van Mil F & Misdorp W 2002 Progesterone receptors in normal, dysplasic and tumourous feline mammary glands. Comparison wit oestrogen receptors status. *Research in Veterinary Science* **72** 153–161

Martin PM, Cotard M, Mialot JP, Andre F & Raynaud JP 1984 Animal models for hormone-dependent human breast cancer. Relationship between steroid receptor profiles in canine and feline mammary tumors and survival rate. *Cancer Chemotherapy and Pharmacology* **12** 13–17

Meuten DJ 2002 *Tumours in Domestic Animals*. Ames Iowa: Iowa State University Press

Millanta F, Lazzeri G, Mazzei M, Vannozzi I & Poli A 2002 MIB-1 labeling index in feline dysplastic and neoplastic mammary leisons and its relationship with postsurgical prognosis. *Veterinary Pathology* **39** 120–126

Millanta F, Lazzeri G, Vannozzi I, Viacava P, Poli A 2002 Correlation of vascular endothelial growth factor expression to overall survival in feline invasive mammary carcinomas. *Veterinary Pathology* **39** 690–696

Misdorp W, Else RW, Hellmen E & Lipscomb TP 1999 *WHO International Histological Classification of Tumors of Domestic Animals. Histological classification of mammary tumors of the dog and the cat. 2nd series, volume VII*. Washington DC: Armed Forces Institute of Pathology

Misdorp W, Romijn A & Hart AA 1991 Feline mammary tumors: a case-control study of hormonal factors. *Anticancer Research* **11** 1793–1797

Misdorp W 1991 Progestagens and mammary tumours in dogs and cats. *Acta Endocrinologica* (*Copenhagen*) **125** 27–31

Morris JS, Dobson JM, Bostock DE 1993 Use of tamoxifen in the control of canine mammary neoplasia. *Veterinary Record* **133** 539–542

Mosselman S, Polman J & Dijkema R 1996 ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Letters* **392** 49–53

Murakami Y, Tateyama S, Rungsipipat A, Uchida K & Yamaguchi R 2000a Immunohistochemical analysis of cyclin A, cyclin D1 and P53 in mammary tumors, squamous cell carcinomas and basal cell tumors of dogs and cats. *Journal of Veterinary Medical Science* **62** 743–750

Murakami Y, Tateyama S, Rungsipipat A, Uchida K & Yamaguchi R 2000b Amplification of the cyclin A gene in canine and feline mammary tumors. *Journal of Veterinary Medical Science* **62** 783–787

Nakopoulou L, Lazaris AC, Panayotopoulou EG, Giannopoulou I, Givalos N, Markaki S & Keramepoulos A 2004 The favourable prognostic value of oestrogen receptor beta immunohistochemical expression in breast cancer. *Journal of Clinical Pathology* **57** 523–528

Nasir L, Krasner H, Argyle DJ & Williams A 2000 Immunocytochemical analysis of the tumour suppressor protein (p53) in feline neoplasia. *Cancer Letters* **155** 1–7

Nelson HD, Humphrey LL, Nygren P, Teutsch SM & Allan JD 2002 Postmenopausal hormone replacement therapy: scientific review. *Journal of the American Medical Association* **288** 872–881

Oonuma T, Morimatsu M, Nakagawa T, Uyama R, Sasaki N, Nakaichi M, Tamamura H, Fujii N, Hashimoto S, Yamamura H, Syuto B 2003 Role of CXCR4 and SDF-1 in mammary tumor metastasis in the cat. *Journal of Veterinary Medical Science* **65** 1069–1073

Owen LN 1980 TNM *Classification of Tumours in Domestic Animals*. World Health Organization, Geneva

Palmieri C, Cheng GJ, Saji S, Zelada-Hedman M, Warri A, Weihua Z, Van Noorden S et al. 2002 Estrogen receptor beta in breast cancer. *Endocrine-Related Cancer* **9** 1–13

Perez-Alenza MD, Jimenez A, Nieto AI & Pena L 2004 First description of feline inflammatory mammary carcinoma: clinicopathological and immunohistochemical characteristics of three cases. *Breast Cancer Research* **6** R300–R307

Martin PM, Cotard M, Mialot JP, Andre F & Raynaud JP 1984 Animal models for hormone-dependent human breast cancer. Relationship between steroid receptor profiles in canine and feline mammary tumors and survival rate. *Cancer Chemotherapy and Pharmacology* **12** 13–17

Preziosi R, Sarli G, Benazzi C & Marcato PS 1995 Detection of proliferatine cell nuclear antigen (PCNA) in canine and feline mammary tumours. *Journal of Comparative Pathology* **113** 301–313

Rhodes DJ 2002 Identifying and counselling women at increased risk for breast cancer. *Mayo Clinic Proceedings* **77** 355–360

Russo IH & Russo J 1987 Biology of disease. Biological and molecular basis of mammary carcinogenesis. *Laboratory Investigation* **75** 112–137

https://doi.org/10.1017/S0022029905001263 Published online by Cambridge University Press

Rutteman GR, Blankenstein MA, Minke JMHM & Misdorp W 1991 Steroid receptors in mammary tumours of the cat. *Acta Endocrinologica* (*Copenhagen*) **125** 32–37

Sartin EA, Barnes S, Kwapien RP et al. 1992 Estrogen and progesterone status of mammary carcinomas and correlation with clinical outcome. *American Journal of Veterinary Research* **53** 2196–2200

Scibelli A, d'Angelo D, Pelagalli A, Tafuri S, Avallone L, Della Morte R & Staiano N 2003 Expression levels of the focal adhesion-associated proteins paxillin and p130CAS in canine and feline mammary tumors. *Veterinary Record* **34** 193–202

Simpson JF & Page DL 1992 Prognostic value of histopathology in the breast. *Seminars in Oncology* **19** 254–262

Sobin LH & Wittekind CH 1997 *International Union Against Cancer* (*UICC*). *TNM Classification of Malignant Tumours. 5th Edn.* New York: Wiley

Speirs V, Malone C, Walton DS, Kerin MJ & Atkin SL 1999 Increased expression of estrogen receptor beta mRNA in tamoxifen-resistant breast cancer patients. *Cancer Research* **59** 5421–5424

Sternlicht MD, Kedeshian P, Shao ZM, Safarians S & Barsky SH 1997 The human myoepithelial cell is a natural tumor suppressor. *Clinical Cancer Research* **3** 1949–1958

Stolwijk JAM, Minke JMHM, Rutteman GR, Hoekstra J, Prop FJA & Misdorp W 1989 Feline mammary carcinomas as a model for human breast cancer. II. Comparison of *in vivo* and *in vitro* adriamycin sensitivity. *Anticancer Research* **9** 1045–1048

Tanabe S, Nakadai T, Furuoka H, Oomachi T, Kobayashi Y, Omata Y, Koyama T, Hondo E, Uzuka T, Sarashina T, Ducusin RJ, Shida T & Dorf ME 2002 Expression of mRNA of chemokine receptor CXCR4 in feline mammary adenocarcinoma. *Veterinary Record* **151** 729–733

Tuck AB, Park M, Sterns EE, Boag A & Elliott BE 1996 *American Journal of Pathology* **148** 225–232

Vail DM & MacEwen EG 2000 Spontaneously occurring tumors of companion animals as models for human cancer. *Cancer Investigation* **18** 781–792

Valavaara R, Tuominen J & Johansson R 1990 Predictive value of tumor estrogen and progesterone receptor levels in postmenopausal women with advanced breast cancer treated with tamoxifen. *Cancer* **66** 2264–2269

Van Garderen E, De Wit M, Voorhout WF, Rutteman GR, Mol JA, Nederbragt H & Misdorp W 1997 Expression of growth hormone in canine mammary tumours. *American Journal of Pathology* **150** 1037–1047

Weijer K & Hart AAM 1983 Prognostic Factors in feline mammary carcinoma. *Journal of the National Cancer Institute* **70** 709–716

Weijer K, Head KW, Misdorp W & Hampe JF 1972 Feline malignant mammary tumours. I. Morphology and biology. Some comparison with human and canine mammary carcinomas. *Journal of the National Cancer Institute* **49** 1696–1704

Wellings SR 1980 A hypothesis of human breast cancer from the terminal ductal lobular unit. *Pathology Research Practice* **166** 515–535

Young B & Heath JW 2000 *Wheater's Functional Histology 4th Edn.* Churchill Livingstone

https://doi.org/10.1017/S0022029905001263 Published online by Cambridge University Press