Canine Mammary Tumours as a Model to Study Human Breast Cancer: Most Recent Findings

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Abstract. *Clinical and molecular similarities between canine mammary tumours and human breast cancer have been described in recent decades. Clinically, the similarities are very strong: spontaneous tumours, hormonal aetiology, age of onset and an identical course of the disease. The clinical characteristics that have an impact on the clinical outcome are also identical: tumour size, lymph node invasiveness and clinical stage. Nowadays, as far as human medicine is concerned, the goal is to identify prognostic factors, mainly at the molecular level, such as those involved in metastasis, which could be used as therapeutic targets to support a better outcome. Moreover, in this area, canine mammary tumours seem to mimic human breast cancer, as a range of similarities are found at the molecular level concerning the overexpression of steroid receptors, proliferation markers, epidermal growth factor, p53 supressor gene mutations, metalloproteinases, cyclooxygenases, among many others. Clinical and molecular data that support canine mammary tumours as a model to study human breast cancer are analysed in this review. Additionally, it is shown that some recent molecular targets in canine mammary tumours may be seen as indicators for similar research to be performed in the corresponding human disease.*

Canine mammary tumours (CMT) have been suggested as a model to study human breast cancer (HBC), for a number of years due to the great number of similarities between them, from epidemiological data to the histological patterns of the neoplastic lesions (1, 2).

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Despite having different lifespans, the average age at onset of mammary tumors is approximately the same for humans (after 40 years) (3) and dogs (after 6 years) (Table I), in addition the reported peak incidence of the disease is also comparable between the two species [humans (50-58 years) and dogs (8-11) (1)].

Among the factors involved in the aetiology of mammary gland tumours, the importance of hormonal influence, both in humans (5) and dogs (6), has long been recognized. The analysis of shared hormonal aetiology in CMT and HBC is only possible if we consider the premenopausal period in women, when the mammary gland is under a cyclic hormonal influence, as in the dog; however, in the female dog there is no menopause.

In this review, we collected information from previous studies that strengthen the value of CMT as models to HBC, as there are many similarities in epidemiological data, prognostic factors and therapeutic targets that may lead to novel advances and improve the efficacy of breast cancer treatments.

Incidence and Risk Factors

Epidemiological studies in the United States (7) and Spain (8) have shown that the incidence of HBC has increased in recent decades, probably due to the greater use and efficacy of screening mammography in the diagnosis of the disease. In dogs, Dobson *et al.* in the United Kingdom, reported a standardized-incidence rate for CMT of 205/100000 dogs/year within a defined population (9), higher than the peak observed for breast cancer in women in the years 2000-2001 by Glass *et al.* (7). This high incidence probably reflects an improvement in the diagnostic efficacy associated with an increased concerning for pet wellbeing by owners. This interest has been rising exponentially. The high number of mammary tumours observed in dogs could be used to a considerable advantage in the performing of clinical trials,

Table I. *The age of medium-sized dogs corresponding to human years [adapted from Metzger, 2005 (4)].*

Dog (years)	Human (years)
6	40-42
7	44-47
8	48-51
9	52-56
10	56-60
11	60-65
12	64-69
13	68-74
14	72-78

applicable in comparative medicine.

The hormonal aetiology is well defined for CMT. Non-spayed female dogs have a 4-fold higher risk of developing mammary tumours compared to those spayed ones before 2 years of age. Male dogs are rarely affected (10). The hormonal aetiology in canines was strengthened by immunohistochemical studies that identified oestrogen (ER) and progesterone (PR) receptors in benign and malignant lesions (11, 12). ERs are more intensively expressed in benign neoplasms or normal mammary tissues than in malignant counterparts (12). In women, the ER status of breast cancer was established as a useful predictor of tumour hormonal dependency and, consequently, the likelihood of obtaining a positive response to an endocrine therapy (13). As in the CMT, in HBC an increase in cellular differentiation with increasing levels of ER and PR was also observed (14). Hormonal dependency is therefore another common feature of the two species.

In canines, some studies suggested an associated risk between obesity and mammary tumours, especially for juvenile obesity. A thin body conformation at 9-12 months of age reduced the risk of breast cancer among spayed dogs by 99%, and among non-spayed dogs by 40% (15). The influence of the diet was also studied, comparing commercial diets to homemade ones (with superior proportions of red meat). The homemade diet was associated with an increased risk of mammary tumours and dysplasia (16). In women, body size is an important predictor of breast cancer survival. The relatively poor survival of heavier women seems only partially explicable by their tendency to have more advanced disease at the time of diagnosis (17). Among women who carry a mutation in *BRCA1* or *BRCA2* genes, those that lost weight between the age of 18 to 30 years had a 34% reduction in the risk of developing breast cancer (18). There is apparently a greater risk among premenopausal women that gain weight after the age of 30 years up to menopause. The biological effect of weight gain may in part depend on the influence of other hormone-related variables, such as puberty, pregnancy, lactation, and menopause (19).

Histological Features

Breast cancer histological type reflects its morphological features and also its biological characteristics. The most common histological type of mammary tumour in women is the invasive carcinoma, more precisely, the invasive ductal carcinoma (20, 21). In the dog, mammary gland tumours can be either malignant or benign and arise from different types of tissues in the mammary gland (epithelial or glandular tissues, and mesenchymal or connective tissues), however the majority of malignant tumours classified as epithelial tumours are carcinomas (22). CMT can be also evaluated on the basis of the degree of nuclear differentiation. As in women, the degree of nuclear differentiation is usually inversely related to the clinical aggressiveness of the tumour (23).

In HBC, histological grading of invasive lobular carcinoma has shown an association with other markers of prognosis and has an independent prognostic value (24). In CMT, histological grading by the method of Elston and Ellis was also related to prognosis, especially in cases of simple carcinoma (25).

Clinical Course

The clinical course of mammary tumours is comparable in dogs and humans. In the dog, the proportion of malignant mammary tumours is reported to be about 50% (26) and approximately 50% of mammary carcinomas metastasize to regional lymph nodes and lungs. Bone metastases are infrequent (27, 26). In the human counterpart, the number of affected axillary lymph nodes is the most important factor for prognosis followed by the TNM status, ER concentration and extent of tumour necrosis (28). Nearly 20% of women with a history of early breast cancer will eventually develop metastases (29). It is noteworthy that in both species, a larger tumour size, presence of lymph node metastases and advanced clinical stage are linked to a worse prognosis (2, 27-32).

CMT could be clinically as aggressive and lethal as the corresponding disease in women. For this reason, in both species, the search for prognostic factors to divide patients into groups with higher risk of recurrence or death due to the tumour and consequently to identify newer therapeutic approaches is required.

Molecular Markers

In recent decades, there has been intense research of new molecular prognostic markers and a remarkable similarity was found between both species. Here we discuss some of these molecular markers, especially those with a particular interest as targets of new anticancer therapies and with potential impact on comparative medicine.

Hormones

Oestrogen receptors. In mammals, steroid hormones play an important role in mammogenesis during puberty. Throughout gestation, full lobulo-alveolar development takes place under the continued stimulation of oestrogen and progesterone (33). The role of steroid hormones and its receptors was firstly studied in HBC during the 1970s. The prognostic implications of ERs were analysed, and a relationship between recurrence after primary mastectomy and a negative ER status was found, without considering the primary size or location of the tumour, number of involved axillary lymph nodes, age or adjuvant therapy (34). In CMT, the presence of ER was correlated with pathological features of the disease, and the presence of ER seems to correlate with the degree of tumoral differentiation (11), similarly to what has been confirmed in humans (13).

There are two known isoforms of the ER, ERα and ERβ. The role of ERα is well established, but further knowledge about the therapeutic implications and prognostic significance of ERβ is still needed. In HBC, it has been confirmed by immunochemistry and Real time-polymerase chain reaction (RT-PCR) that ERα is the major ER (35). In CMT, a lower ERα expression was related to a worse prognosis, concurrently in tumours with a larger size and skin ulceration (36).

With respect to ERβ, its expression was described in HBC and was demonstrated to be related to an increased survival time in those patients treated with tamoxifen and with ERα negative tumours (37). In CMT, ERβ-positive tumours are more frequently benign than malignant. The higher expression of ERβ in malignant CMT with a lower grade of malignancy is suggestive of its value as a factor of good prognosis (36). These differences in ERs have a considerable influence on endocrine therapy and so selective ER modulators (SERMs), which work as complete antagonists for ERα and agonists for ERβ, may be useful in the treatment of CMT as was suggested for HBC, especially in chemoprevention strategies (38).

Progesterone receptors. PRs play a role in breast development, promoting the terminal ductal lobular units expansion during puberty and pregnancy (39).

In HBC, PRs were found to be valuable indicators of recurrence, as a negative PR status is related to a worse prognosis (40, 41). The combined ER and PR status has revealed differences in overall survival, with both ER$^-$ and PR- status having the worst survival time, followed by ER$^-$/PR$^+$, ER$^+$/PR$^-$ and ER$^+$/PR$^+$ (42).

In CMT, normal unaltered and benign tissues commonly contain both ER and PR (12). Differences between benign and malignant tumours were found regarding the simultaneous immunohistochemical expression of PR and ERα. Among benign tumours, the most common receptor status of positivity was ERα$^+$/PR$^+$, while among malignant tumours, the ERα$^-$/PR$^+$ status was twice as common as the double positive (34). As the disease progresses towards metastasis, malignant tumours have a tendency to lose their hormonal dependency, therefore malignant tumours and their metastases tend to be negative for PR and ER (12). As in women, the ER$^-$/PR$^-$ profile seems to indicate a worse prognosis.

Growth hormone (GH) and insulin-like growth factor-1 (IGF-I). The role of the GH/IGF-I axis in human breast tumourigenesis has been investigated (43-45). GH-induced IGF-I is required to permit estrogen and progesterone action in the mammary gland. Prevention of preneoplastic breast disease and breast cancer by GH/IGF-I inhibition is a recent concept (44). In canines, it was observed that local production of GH by mammary gland tissue was induced by progestin stimulation. It was postulated that this autocrine GH might be the mediator of the epithelial hyperplasia classically observed after progestin administration (46). Soon after mammary gland biosynthesis of GH measured by *in situ* hybridization and immunoelectron microscopy was described (47). However, the role of progestins in triggering GH production in normal and neoplastic mammary gland remains to be clarified.

The molecular characterization of the canine full-length GH receptor (GHR) in canine mammary tissue revealed extensive homology with the GHR sequence of several species, including that of humans (48), which might support a more important role of this hormone in comparative pathology.

Recent data has shown a strong correlation between tissue levels of progesterone and GH in CMT homogenates, suggesting that the progesterone/GH axis plays a distinct role in CMT (49). The GH/IGF-I axis seems also to be implicated in the prognosis of CMT as pre-surgical serum concentrations and tissue GH and IGF-I content of the malignant tumours are related to reduced post-surgical survival times (50). The same study reported that serum IGF-I concentrations in female dogs with malignant tumours were significantly higher than those in healthy controls (51). For the first time, in both human and veterinary medicine, the prognostic value of GH and IGF-I in mammary tumours was demonstrated. This evidence could open a window to new therapeutic modalities, namely involving the use of GH competitive antagonists such as pegvisomant (52, 53).

Prolactin. Prolactin, well known for its lactogenic activity, may also play a role as growth factor in mammary cancer. In HBC, the hormonal dependence on prolactin was suggested by the improved clinical condition of patients after hypophysectomy and the increased survival of mammary tissue cultures with prolactin supplementation (54). HBC cells and tumours co-express prolactin and sex steroid

receptors and their expression levels were found to be cross-regulated. This fact might explain the synergy between prolactin and the sex steroid hormones during mammary development and in neoplastic growth, especially that between progesterone and prolactin (55).

In CMT, it was firstly proposed in 2005 (50) that steroid hormones and prolactin act as local growth factors in malignant tumours, stimulating their proliferation. Furthermore, it has been observed that levels of prolactin in tissue homogenates were correlated with the clinical tumoral characteristics studied, presenting a strong correlation with steroid hormone levels. Further studies on the influence of prolactin on prognosis are still lacking in veterinary medicine.

Molecular markers for cancer. With regard to molecular oncology and the fundamental gene alterations in order to the development of tumours, numerous antigens and oncogenes have been identified in the last decades. From the wide range of genes involved in breast cancer, some of them were also found to play a role in the carcinogenesis in CMT. In order to unravel the complex molecular pathogenesis of breast cancer, research on CMT contribution is enlightening new paths towards possible answers.

Proliferation markers. The antigen Ki-67 is a nuclear protein actively expressed in cycling cells, but not after mitosis (56). Ki-67 labelling has been found in a wide variety of tumours, both in humans (57-59) and dogs (60-62).

In humans, immunohistochemical studies with the antibody Ki-67, as measure of tumour cell proliferation, suggest that it is a useful prognostic indicator for the relapse-free period in patients with breast carcinoma (63). Other proliferation markers have been studied along with the Ki-67 antigen, such as proliferating cell nuclear antigen (PCNA). Several authors also described a reliable prognostic value for PCNA (64-70), although a poor value for this prognostic marker in these tumours was also reported (71).

In the canine counterpart, the immunohistochemical expression of Ki-67 and PCNA was studied and a relation with a poor prognosis was established for Ki-67 (61). Malignant CMT also had significantly higher PCNA index than normal mammary gland, hyperplasia and benign tumours (72). Ki-67 expression in benign and malignant tumours, similar to that of HBC, was reported, as was a significant association with the appearance of distant metastases, and a relationship between the PCNA index and histological criteria of malignancy (73).

p53 tumor suppressor gene. The p53 tumor suppressor gene plays a major role in controlling cellular growth after DNA damage. When mutations happen in this gene, deregulated cell proliferation occurs, which underlies tumour formation and progression (74). In HBC, immunoexpression of accumulated *p53* nuclear protein, the result of *p53* gene mutation, seems to have prognostic value, being strongly correlated with poor patient survival (75, 76). In CMT, mutant p53 expression is also recognized for its prognostic value (77-79). The frequency of *p53* mutation in CMT (20%) is similar to that observed in HBC (75, 77, 80). Likewise, mutations in the conserved domains of p53 appear to play a significant role in mammary carcinogenesis in both humans and canines (81). Veldhoen *et al.* (82) identified *p53* mutations in CMT located within exons two, four and five, and a point mutation on Ala125Val, corresponding to the Ala125Val mutation of the human *p53* gene. This finding in an aggressive carcinoma is suggestive of an association between the aggressive type and *p53* mutations, as reported previously in HBC studies. The organization of canine *p53* coding exons and gene products are very similar to those reported in the human gene, suggestive of a shared therapy target (80).

p63. The *p63* gene belongs to the *p53* gene family; even though it is not a tumoral suppressor, it is involved in epithelial stem cell regeneration. The analysis of *p63* in varied types of human cancers has shown overexpression, particularly in basal epithelial cells (83). There is still the possibility that *p63* gene acts as a tumour promoter under certain pathological conditions (84).

In humans, p63 is overexpressed in the basal phenotype of breast carcinomas; this fact supports its role in differentiation and growth control in stratified epithelia (85). In HBC, p63 appears to be a sensitive and specific marker for myoepithelial cells and, due to the intense staining observed for papillary carcinomas, myoepithelial differentiation is suggested within this type of neoplasm (86). In canine mammary tissues, p63 has proven to be a potential myoepithelial cell marker, useful in diagnosis to distinguish basal or myoepithelial cells from stromal myofibroblasts (87). It has also been verified that p63 overexpression suggests the possibility of a closer relationship involving myoepithelial and stem cells, thus clarifying the histogenesis of CMT (87).

HER-2/neu. *HER-2/neu* is an intensively studied proto-oncogene in human medicine, and has become a widely used prognostic factor, with excellent treatment results being obtained using specific therapy for HER2/neu+ tumours (88). In veterinary medicine, studies on the same oncogene have not been so abundant. One study performed by Rungsipipat *et al.* (89) analysed 79 CMT. HER2/neu expression was present in about 50% of the benign tumors, such as adenomas of the simple and complex types, and in 19.1% of the adenocarcinomas. The percentage of malignant CMT expressing HER2/neu in that study was of the same order as that of HBC (20% to 30%) (90). In the study of Kerns and

collaborators (90), co-expression of p53 and HER2/neu was also detected, which could be interpreted as the loss of one mechanism that controls cell proliferation, activating malignant cell potential.

As in humans (91-93), four distinct phenotypic subtypes were identified in malignant CMT, based on the immunohistochemical expression for HER2 and ER: luminal A (ER$^+$/HER2$^-$, 44.8%), luminal B (ER$^+$/HER2$^+$, 13.5%), basal-like (ER$^-$/HER2$^-$, 29.2%) and HER2 overexpressing (ER$^-$/HER2$^+$, 8.3%), with individuals with the basal-like subtype having shorter survival (94). This serves as a proof of the molecular heterogeneity of CMT and its potential as a model of breast cancer oncogenesis.

Epidermal growth factor receptor. The epidermal growth factor receptor (EGFR), also known as HER-1, is related to the previously referred tyrosinase receptor HER2/neu. The EGFR is suggested as a molecular marker of prognosis in cases of triple negative HBC (ER$^-$, PR$^-$ and HER2/neu$^-$) in which there is a lack of specific therapeutic targets (95). In CMT, a tendency toward shorter disease-free survival and overall survival was found in dogs with positive EGFR expression. Furthermore, similarly to reports from previous HBC studies, myoepithelial cells in normal, hyperplastic and benign lesions consistently express EGFR (96). Concurrently high progesterone and EGF tissue concentrations suggest that a mechanism involving the progesterone sensitization of neoplastic cells to proliferative and angiogenic effects of EGF could be present in CMT (97).

BRCA genes. BRCA2 gene plays an important role in the maintenance of genomic stability and is the basis of genetic predisposition for breast cancer. *BRCA2* gene products modulate the activity of RAD51, regulating the expression of recombinase, a protein essential for the maintenance of genome integrity through DNA repair and for growth by homologous recombination (98).

In HBC, low immunoexpression of the BRCA1/BRCA2/RAD51 complex is correlated with high histological grade of the tumour, suggesting an association between loss of these proteins and an aggressive tumour phenotype (99). In lymph node metastasis of canine mammary adenocarcinomas *Rad51* mRNA was mostly overexpressed, while *BRCA2* was only expressed in 50% of the cases. Rad51 might be associated with malignancy of CMT, but to confirm this hypothesis further studies are still required with a large series of tumours (100).

Uva *et al.* (101) studied the expression pathways of oncogenes shared in human and canine breast cancer and found a great similarity between the networks of signalling circuitries that govern the biology of mammary cancer, as well as a strong correlation between most of the prognostic signatures in both species. The possibility of the development of transcriptional biomarkers in dogs to be applied subsequently to humans was also proposed (101).

Matrix metalloproteinases. Matrix metalloproteinases (MMP) are a family of zinc-binding enzymes that cleave matrix components; they contribute to tumour cell invasion by the proteolysis of the extracellular matrix which might have a boundary effect for tumour cells (102).

In HBC, the evaluation of MMP-2 and MMP-9 by zymography reported a significant correlation of MMP-2 activation with lymph node metastasis and suggested that MMP-2 could be useful as a drug target in the treatment of breast cancer (103).

In CMT, the immunohistochemical expression of MMP-9 and MMP-2 revealed a higher intensity for MMP-9 expression in malignant tumours, which suggests that MMP-9 plays an important role in the aggressive behavior of these tumours and indicates its possible prognostic implication (104). Another study, performed among varied canine neoplasms, has shown that in zymography and immuno-histochemistry, MMP production was higher at the edge of canine malignancies compared with the tumour centre, in accordance with the previously suggested role of MMPs in human cancer invasion (105).

Phosphatase and tensin homolog. Phosphatase and tensin homolog (*PTEN*) is a gene often lost in late-stage tumours. Recently, the mechanisms implicated in its regulation have been increasingly studied, and its role in human diseases such as cancer and diabetes is now better understood (106). In HBC, the loss of PTEN protein expression is relatively common and is apparently linked to an aggressive ER$^-$/PR$^-$ phenotype, lymph node metastasis, and poor survival time (107). In CMT, the percentage of cases of loss of PTEN protein expression is similar to that reported in humans (108). Additionally PTEN loss in invasive carcinomas is higher when compared with that of *in situ* lesions, suggesting that it is a phenomenon occurring in advanced phases of carcinogenesis as previously reported for human breast cancer (108). Another study in CMT shows an direct correlation between clinicopathological stage and loss of PTEN expression, thus suggestive that the altered expression of PTEN might underlie molecular mechanisms of differentiation (109).

Heat-shock proteins. Most heat-shock proteins (HSPs) have strong cytoprotective effects and behave as molecular chaperones, having properties in protein homeostasis; their function is to restore the balance after the activation of signalling pathways for acute or chronic stress both in humans and dogs (110). HSPs play a double role: while HSPs accumulate in cancer cells and contribute to tumour survival, on the other hand, they also have an extracellular

immunological function that can be used to induce a specific anti-tumoural response (111). A study involving both CMT and HBC showed an enhanced expression of Bcl-2, Bcl-XL, and HSP-70 and -90 (related to the inhibition of apoptosis) with down-regulation of Bax and caspases, which are known to regulate apoptosis (112). The escape of neoplastic cells from apoptosis allows them to sustain mutations and survive for longer, being a characteristic aspect of carcinogenesis. Taken together, these findings reinforce the value of the canine model in understanding the molecular mechanisms of carcinogenesis.

Mucins. Mucins are a family of proteins that have a common glycosylated domain. Mucins have been identified as markers of adverse prognosis and as attractive therapeutic targets, as they promote tumour progression (113). In HBC, the presence of mucin 1 (MUC1) has been related to well-differentiated tumours and to an improved prognosis (114). MUC1 expression can be seen on the apical membrane of tumour cells in mucinous carcinomas, or more strongly in the cytoplasm of invasive ductal carcinomas (115). Correspondingly, different patterns of MUC1 expression are also present in malignant CMT and normal adjacent mammary gland tissues. There is an apical localization, resembling the normal mammary gland in humans, hence suggesting a comparable biological function (116); in this recent study, MUC1 overexpression was associated with distant metastasis and thus may have a role as a potential prognostic marker or immunotherapeutic target in these neoplasias.

Maspin. Maspin is a serine proteinase inhibitor that has suppressor activity and is expressed in human normal mammary epithelia, however, to date there are limited data on its clinical significance. At very low concentrations, maspin is capable of inhibiting the invasion of breast and prostate cancer cells by blocking their motility at the cell surface (117). In HBC, the immunohistochemical expression of maspin was proven to be significantly correlated with an aggressive phenotype in relation to higher histological grade, a larger tumour size and negative PR status (118). In the canine mammary gland, maspin seems to be a sensitive myoepithelium marker. Nevertheless, stromal myofibroblasts did not react with maspin antibody, which could represent an advantage in detecting *in situ* and microinvasive forms of malignant CMT with a myoepithelial cell origin (119). However, studies of the prognostic significance and biological aggressiveness of tumour as related to maspin expression are still lacking in CMT.

Sialyl Lewis x (sL^x) antigen. Sialyl Lewis x (sL^x) antigen is a ligand for E-selectin, responsible for adhesion of several types of human carcinoma cells to endothelium, and it was suggested that these interactions influence the formation of metastases in colon and pancreatic cancer (120). Renkonen *et al*. (121) observed that in breast carcinomas, the expression of these oligosaccharide epitopes was enhanced in metastases compared with primary lesions, probably being involved in interactions with endothelial selectins during metastasis formation. On the other hand, sL^x expression in breast cancer does not seem to be related to distant metastasis and poor prognosis, although a higher expression of sL^x was observed on the surface of high-grade ductal carcinoma *in situ* cells, while expression was lower in the surrounding invasive tumor. This pattern resembles that of CD31 expression, which is used as a marker of neoplastic angiogenesis (122).

The majority of CMT express sL^x, through immuno-histochemical analysis, while in the normal mammary gland, sL^x expression is absent. Nevertheless, no relationship with clinicopathological features or prognosis was observed (123). Pinho *et al*. (124) found a significant correlation between sL^x expression and lymph node metastasis in malignant CMT, which emphasizes the role of sL^x antigen on local lymphatic invasion and metastasis. However, since no association with distant metastasis was found, the authors conclude that sL^x might not contribute to the haematogenous pathway of metastasis (124).

Cyclooxygenase-2. There is accumulating evidence suggesting the role of cyclooxygenase (Cox), particularly Cox-2, in tumour development and progression both in human and canine cancer (125).

Cox enzymes are the main targets of non-steroidal anti-inflammatory drugs. The first studies about Cox-2 expression in HBC were published in 1998 (126); in 2003, the first evidence of Cox-2 expression in CMT arose (127). In HBC, the overexpression of COX-2 was thought to be involved in the production of prostaglandins, during tumourigenesis (126) and seems to be implicated in the first steps of mammary carcinogenesis (128). Larger and metastasizing tumours preferentially express COX-2; accordingly, COX-2 expression showed a strong prognostic impact for disease-free survival and overall survival. (129-133). Interestingly, similar findings were observed in CMT. Several studies showed that malignant tumours expressed more Cox-2 than did benign neoplasias (134-137) and there is a correlation between higher Cox-2 levels and reduced disease-free and overall survival times (138).

In HBC, an association was demonstrated between COX-2 overexpression and angiogenesis using factor VIII-related antigen (133) and the anti-CD31 antigen also known as platelet endothelial cell adhesion molecule (PECAM-1) (139). This supports the role of COX-2 in tumourigenesis and provides support for a potential therapeutic role for COX-2 inhibitors, due to their antiangiogenic properties. In the canine counterpart, the relationship between Cox-2 was also explored in mammary tumours, presenting a correlation with microvessel density and vascular endothelial growth factor (VEGF) overexpression (140).

It is noteworthy that in malignant CMT, the highest levels of Cox-2, assessed by enzyme immunoassay, were found in inflammatory mammary carcinoma (IMC) (134), a very aggressive CMT, with histological and clinical similarities with human inflammatory breast cancer (IBC) (141, 134). In both species, inflammatory cancer is rare, but some empirical evidence suggests that there has been an increase in this type of cancer in recent decades. Curiously, there are no reports of COX-2 expression in IBC and the possible use of COX-2-selective nonsteroidal anti-inflammatory drugs as co-adjuvant in its treatment, although these have already been suggested as chemopreventive therapy to reduce the risk of breast cancer in general (125). As for other markers that revealed strong similarity between CMT and HBC, it is expected that IBCs also express elevated amounts of COX-2 like their canine counterpart. In the Authors' opinion, this topic deserves additional research for a better understanding of this aggressive disease.

Future Perspectives

Nowadays, one of the major goals in human and veterinary medicine is to identify prognostic factors that could identify therapeutic targets to support a better outcome and to improve survival. In HBC, a great number of prognostic markers have been identified, however, only a few are routinely used to group patients and choose the most adequate therapeutic approach. In CMT, the use of prognostic markers is still reserved for research purposes.

Usually, investigations performed in CMT follow similar research previously performed in HBC. However, there have been some exceptions, as sometimes the investigations performed in CMT might enlighten newer research themes in human disease. Such is the case for Cox-2 research in IMC, which was firstly studied in CMT (134), and in the investigation concerning the prognostic value of GH/IGF-I, previously mentioned (49).

Taken together, the accumulating similarities presented here reinforce the notion that it is of great interest to consider CMT as a resource to enhance the understanding of molecular pathogenesis of HBC. It remains for us to take advantage of this widely available resource for the research of new biomarkers needed for earlier detection and more complete biochemical characterization of breast cancer, and for the development of improved therapeutic strategies.

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