IN VITRO COMPARATIVE MODELS FOR CANINE AND HUMAN BREAST CANCERS

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Abstract

During the past four decades, an increased number of similarities between canine mammary tumors and human breast cancer have been reported: molecular, histological, morphological, clinical and epidemiological, which lead to comparative oncological studies. One of the most important goals in human and veterinary oncology is to discover potential molecular biomarkers that could detect breast cancer in an early stage and to develop new effective therapies. Recently, cancer cell lines have successfully been used as an in vitro model to study the biology of cancer, to investigate molecular pathways and to test the efficiency of anticancer drugs. Moreover, establishment of an experimental animal model for the study of human breast cancer will improve testing potential anti-cancer therapies and the discovery of effective therapeutic schemes suitable for human clinical trials.

In this review, we collected data from previous studies that strengthen the value of canine mammary cancer cell lines as an in vitro model for the study of human breast cancer.

Keywords: canine mammary cancer, canine model, human breast cancer, cancer cell lines

Introduction

Mammary tumors represent one of the most common types of gynecologic neoplasia diagnosed in women and female dogs [1,2]. The results from previous clinical studies have reported an incidence of approximately 50% in malignant canine mammary tumors [3,4]. In the case of both species, human and canine, malignant mammary tumors may relapse after surgical excision and metastasize to distant organs such as lymph nodes, lung, or liver [5].

Comparative oncological studies from the past four decades have successfully used canine mammary tumors as a potential suitable model for studies in human breast cancer research [6,7,8,9] due to the considerations based on the similarities from the clinical and molecular aspects [10,11,12] from epidemiological data to the histological

patterns of the neoplastic lesions [13,14,15,16,17] and for the morphological and biological behavior similarities [17,18,19,20,21,22]. From the clinical point of view, the similarities are very strong: spontaneous tumors, intraepithelial breast lesions, hormonal etiology, age of onset and an identical course of the disease. Among the etiologic factors involved in breast cancer carcinogenesis, the importance of hormonal influence both in humans [23] and dogs [24] has been recognized.

Given the large number of cellular events involved in cell growth, differentiation, proliferation, invasion and metastases [25], the investigation of multiple molecular alterations in concert has assumed great importance due to the introduction of high-throughput technologies [26].

Moreover, because of the high mammary cancer incidence, the spontaneous and heterogeneous nature of the mammary tumor development, the same environmental risks, the domestic dog has been suggested as a valuable breast cancer model for studies of preclinical research [27,28].

MacEwen (1990) supported the claim that spontaneous canine mammary tumors represent a suitable model for the study of human breast cancer therapy and biology. Nevertheless, the potential role of the domestic dog has not been clearly determined in human breast cancer research and it represents an issue that still needs to be debated [8].

Molecular similarities

The similarities between human and canine species found at the molecular level involve the overexpression of the steroid hormone receptors (estrogen, progesterone, androgens), proliferation markers (Ki67, AgNor), epidermal growth factor (EGF), p53 suppressor gene mutations, metalloproteinases, cyclooxygenases, among many others. Recently, a molecular-based classification of human breast has been established, which seems to be a better tool than using the morphological characteristics to establish precise similarities between these two species. In the case of canine mammary tumors, it is well known that the loss of hormone receptors plays an important role in tumor progression [29,30] and the overexpression of ERB-B2 products leads to malignancies [31,32].

Histological and morphological similarities

Canine mammary tumors can occur in multiple sites and may vary in histology within or among different tumor sites in an individual. These tumors usually possess a complex morphology represented by epithelial, mesenchymal or mixed-type cells. In general complex adenoma and benign mixed tumor are the most common histological type, carcinoma is the most malignant form, while pure benign mesenchymal tumors are rare [33]. Sarcomas and malignant mixed tumors are more common in dogs than in other species [3].

To make any comparisons feasible there is a need to take into consideration several differences between the mammary tumors of these two species. Myoepithelial cell proliferation is a frequent finding in the so-called complex and mixed patterns of canine mammary tumors [34], but it is an uncommon feature of breast cancer in women [35].

Epidemiological similarities

The location and incidence of different tumor types depend on a number of variables, predominantly age, breed, sex and geographic location. Generally dogs develop very similar cancers at sites very analogous to those found in humans. The malignancies that represent a practical use for therapeutic studies are osteosarcoma, mammary neoplasia, oral melanoma, oral squamous cell carcinoma, nasal tumor, lung cancer, soft tissue sarcomas and malignant non-Hodgkin's lymphoma [8].

Breast cancer also occurs when the receptors of steroid hormones are excessively expressed, the apoptosis process becomes ineffective or the cell cycle is dysregulated [36]. Immunohistochemistry studies tried to establish immunohistochemical markers for canine mammary tumors as it follows: p63 and vimentin have been used to define myoepithelial cells [37], cytokeratins have been used for epithelial tumors with stratified and squamous origin [38,39,40]. Gilles et al. (1999) [41] and [42] Kokkinos et al. (2007) observed that vimentin expression is increased in aggressive breast cancer. Gilles et al. (2004) discovered that metalloproteinases which have been reported to be markers of malignancy in human breast cancer are also involved in tumor development, angiogenesis, invasion of the basement membrane and stroma and metastasis of canine mammary tumors [43]. Lee et al. (2004) associated the frequency of TP53 mutation with a high grade of malignancy and a poor prognosis in the case of canine mammary tumors [44]. A study made by Ahern et al. (1996) correlated HER-2/neu overexpression in canine mammary tumors with different grades of malignancy [32]. Osborne (1999) observed that mammary tumors with the absence of estrogen receptor expression were associated with an increased chemoresistance both in human and in canines [45].

Despite having different lifespans, the average age at the onset of mammary tumors is approximately the same for humans (after 40 years) [46] and dogs (after 6-7 years) [47]. In addition the reported peak incidence of the disease is also comparable between the two species [humans (50-58 years) and dogs (8-11) years)] [9].

Hormonal similarities supporting cancer development

In both species the mammary neoplasm may also occur in a hormonal-dependent manner and in the case of canines the development of mammary tumors is highly correlated with steroid sex hormones (progesterone exposure representing the main risk factor) [48,49]. Through immunohistochemical analyses it has been demonstrated the expression of estrogen and progesterone receptors in canine mammary tumors has been shown, but

their prognostic significance is yet to be established [50].

The risk of developing a mammary cancer in sterilized female dogs prior to the first estrus is 0.05% and increases up to 26% if sterilized after the forth estrus [49]. Early pregnancy and early ovariectomy in women lower the incidence of developing a breast tumor. In studies performed by MacEwen et al. (1982) [51] and Martin et al. (1984) [52] it has been proven the expression of both estrogen and progesterone receptors in the case of canine mammary gland. These receptors have been demonstrated to be present in approximately 70% of benign mammary tumors and between 50% and 60% of malignant mammary tumors expressing either estrogen or progesterone receptors. In the case of human mammary neoplasias, up to 60% express estrogen receptors. The malignancy of mammary tumors in female dogs ranges between 41% to 53% [18].

The most frequent histological types of epithelial tumors are carcinomas divided into simple, complex, adenocarcinoma and solid carcinoma. By evaluating the degree of nuclear differentiation, the tumors can be classified as poor, moderate and well differentiated. It has been demonstrated that when a tumor develops is less differentiated, tends to lose its sex steroid hormone receptors and turns into a more aggressive type [51]. Both human and canine species share some common breast cancer characteristics like predominance of carcinoma, metastatic pattern, hormonal dependency and tumor development. Because of the multiple similarities mentioned previously, canine mammary neoplasms seem to represent an appropriate model for the study of human breast cancer biology and molecular mechanisms associated with the response to therapy.

Given the fact that dogs develop naturally, and in large part, the same histologically type of mammary tumors as humans with an intact immune system and with a syngeneic host and tumor microenvironment, they are considered to be an excellent model for human breast cancer studies. Furthermore, in the case of both species the environmental, age, sex, and reproductive factors that lead to cancer progression and development are identical. Furthermore, because of the similar role of the P450 cytochrome in both species, the canine model could be used in clinical trials for testing human anticancer drugs [53].

The use of cancer cell lines in breast cancer research

A recent approach in cancer research is the use of tumor cell lines as an in vitro model for the study of different neoplasias which requires particularly genetic analyses. Previous reports on characterized cancer cell lines have shown that they are also excellent in vitro models for the study of cancer biological mechanisms [54]. By using characterized tumor cell lines, it was concluded that it was an easy way to identify molecular pathways and deregulated genes involved in some types of cancers (ex. lung, breast) [55,56]. Moreover, some original cell models

have been created to test the efficiency of anticancer drugs [57,58,59]. In the last decade, cancer cell lines were used for testing and developing anticancer therapies [60]. In the late 1980s, the US National Cancer Institute developed a large anticancer drug screening by inoculating into animal specimens over 60 types of human cell lines for testing chemotherapeutics [61].

Concerning breast cancer research, there is an essential need to find an appropriate in vitro model. The potential uses of different tumor cell lines as models for cancer research are to prospectively investigate some genetic and epigenetic alterations as well as the cellular pathways [54], to investigate processes like deregulation in cell proliferation, apoptosis, and cancer progression [56], to identify potential molecular biomarkers [62], likewise to screen and characterize potential new cancer therapies [58,63]. According to a study made by Van Staveren et al. (2009), the use of experimental models for cancer research is necessary for the following reasons: to reproduce the physiopathological evolution of the cancer from its origin to the advanced stages, to investigate hypotheses related to the origin, pathogenesis, pathobiology and physiopathology of human tumors, to perform studies on molecular mechanisms, to screen and test chemical compounds as potential therapeutic targets, to identify molecular biomarkers [62].

During the last decade, breast cancer cell lines started to become the most widely used model for investigating processes involved in breast cancer carcinogenesis like proliferation, apoptosis and migration [64]. Thus, by using the cancer cell line model the results concerning the genes and signaling pathways that regulate these complex processes can be relatively quick and easy to obtain. Established cell lines are easy to multiply, relatively facile to genetic manipulation (i.e plasmid transfection) and, under standardized experimental conditions, the results obtained can be relatively fast and generally reproducible and quantifiable.

In vitro canine models for the study of mammary cancer

Nowadays, there is only one standardized canine mammary cancer cell line, REM 134 available at Cell Culture Collections from Public Health England [65]. REM 134 mammary carcinoma cell line was firstly characterized in 1982 by Professor Else et al. [66]. This cell line exhibited tumorigenic properties when inoculated subcutaneously into nude mice. However, in 1986 Wolfe et al. established five epithelial cell lines (CMT-1, CMT-2 ,CMT-3, CMT-4 and CMT-5) from cultured canine mammary carcinomas and one myoepithelial cell line (CMT-6) [67]. Moreover, in 1989 a group of researchers lead by van der Burg isolated cell lines from metastases of estrogen receptor-negative canine mammary carcinomas [68]. Three years later, Professor Dr. Hellmén Eva isolated and performed a detailed cytogenetically characterization on five dogs with

spontaneous mammary tumors of the following cell lines: CMT-U27 (ductal invasive carcinoma), CMT-U111 (lobular invasive carcinoma), CMT-U155 (noninvasive ductal carcinoma), CMT-U131 (infiltrating ductal carcinoma of scirrhous type) and CMT-U229 (atypical benign mixed tumor) [69].

Proteomics analyzes

Proteomics is a very efficient technique used for the identification of protein pattern changes in a large variety of diseases, including neoplastic disorders [70]. By using proteomics techniques, a tumor-specific proteomic profile for a certain pathology can be established, which will increase the discovery of novel biomarkers with predictive and prognostic value for a more accurate diagnosis. In addition, proteomics techniques could provide an important tool for understanding the tumor's pathogenesis [71,72]. In human neoplasias, proteomics analyses are being routinely used, but in the case of canines which serve as an excellent spontaneous model for the study of human breast cancer biology they are rarely used for cancer investigation. In recent years, another important focus in cancer research is represented by tumor protein profiling, hence cancer cell lines are regarded as a suitable tool for proteomics analyzes since they hold an important source of protein.

The heterogeneity of solid tumors represented by the mix of structural elements including epithelial cells, vascular and stromal structures, nerves and inflammatory components [73,74] is a major problem in analyzing the protein profile using 2-dimensional electrophoresis method (2-DE) and subsequent mass spectrometry. However, the proteomics researchers are determined to use cell lines instead of tissue samples [75,76,77,78,79,80] because they confer a more homogeneity to the protein lysate.

In breast cancer research, the aim of using the immunoproteomic analysis is to identify new biomarkers for early tumor detection, diagnosis, and prognosis. Several human breast cancer cell lines including MCF-7 and SUM-44 were used to obtain protein lysate containing tumor antigens like tubulin, the haptoglobin-related protein, HSP60, the tumor suppressor prohibitin, peroxiredoxin-2, and RS/DJ-1 that was noted to induce a humoral immune response [81,82].

In 2013 Zamani-Ahmadmahmudi et al. made the first study that used two-dimensional gel electrophoresis technology for protein profiling of a canine mammary cell line [83]. By immunocytochemistry analysis it was shown that this new isolated canine mammary cancer cell line expressed the estrogen receptor, pancytokeratin, cytokeratin-low (CK8, CK14), vimentin, and negative for progesterone receptor and cytokeratin-high (CK18). The results from the 2-DE proteome analysis of the new established cell line confirmed that it is suitable for canine mammary proteomics studies. So far there is no standardized canine mammary tumor cell line that might serve for proteomic analyses.

The role of Cyclooxygenase-2(COX-2) in breast tumor models

Cyclooxygenase-2 (COX-2) is an important enzyme involved in prostaglandins (PGs) biosynthesis. Prostaglandins are lipid mediators that have been reported to be involved in tumorigenesis and one of the essential step in their synthesis is mediated by the enzyme prostaglandin G/H synthase also known as cyclooxygenase (COX) [84,85]. A number of studies demonstrated that overexpression of COX-2 is involved in human breast cancer [86,87,88,89,90].

The immunohistochemical studies performed by Doré´ et al. (2003) [91] and Heller et al. (2005) [92] revealed that COX-2 was overexpressed in canine mammary cancers. Likewise the overexpression of COX-2 has been reported in several types of human cancer including breast and targeted therapy on COX-2 raises the possibility of a promising cancer prevention and treatment [93,94,95].

In 2006, Brunelle et al. investigated for the first time the expression, regulation and possible role of COX-2 overexpression in five canine mammary epithelial cell lines obtained from mammary tumors (CF33, CF41, CMT9, CMT12, and CMT28) and one cell line (CF35) isolated from a normal canine mammary gland. Results indicated that COX-2 protein was strongly overexpressed in the CMT12 canine mammary neoplastic cell line, whereas low levels of COX-2 were identified in the rest studied cell lines [96].

These studies suggest that overexpression of COX-2 could play an important role in canine mammary carcinogenesis.

Differential response to chemotherapy in inflammatory breast/mammary cancer

In a study made by Hsiao et al. (2014) two new canine mammary cell lines: DTK-E and DTK-SME were established and characterized from a single malignant CMT tumor [97]. Their results showed that both cell lines had heterogeneity in cell morphology, protein marker expression, tumorigenicity, and chemoresistance, suggesting that DTK-E and DTK-SME established cell lines may represent a useful experimental model for the study of mammary cancer, especially in investigating tumorigenesis and screening for potential anticancer drugs.

Inflammatory mammary cancer (IMC) and inflammatory breast cancer (IBC) represent a subtype of one of the most aggressive mammary neoplasia that affects female dogs and humans and share some epidemiological, clinical and histopathological features [98,99,100]. In the case of both species, this subtype of cancer has been proven to be highly angiogenic and angioinvasive [101,102,103,104]. Moreover, in the case of both species, the main histological feature of this neoplasia is the massive invasion of dermal lymphatic vessels by neoplastic cells which block lymph drainage causing the specific edema [98,104,105]. In vitro studies on different established subtypes of mammary

cancer cell lines have been conducted in order to facilitate the understanding of some important processes in breast cancer development like angiogenesis, progression and tumor growth [64,106,107]. Recently, several research studies performed by Majchrzak et al. (2013), Hsiao et al. (2014), Król et al. (2014) managed to establish new canine mammary carcinoma cell lines, but none of them includes a canine inflammatory mammary cancer cell line [97,108,109].

In 2015 Caceres et al. established IPC-366, the first inflammatory mammary cancer cell line with an epithelial basal phenotype, negative for the expression of ER, PR, HER2 and also highly proliferative in vitro and in vivo. This new established IMC cell line maintains the histological features of IMC in vivo and exhibits vasculogenic mimicry in vitro and in vivo.

Given the fact that inflammatory mammary cancer shares epidemiologic, histopathological and clinical characteristics with the human inflammatory breast cancer, the use of this new canine inflammatory mammary cancer cell line is to develop comparative oncological studies for investigating possible anticancer therapies for this aggressive pathology [110].

More than 33 human breast cancer cell lines have been established which derived from primary tumors, metastatic tumors and pleural effusion [64,106,111]. Nonetheless there are few cell lines available for performing studies on the biology of IBC: SUM149 (ER/PR-, HER2-), SUM 190 (ER/PR-, HER2+), MDA-IBC-3, KPL4 and WIBC-9 (all ER/PR- and HER2+) and a recent characterized cell line FC-IBC02 [112,113].

The role of cancer stem cells in tumor development

Cancer stem cells (CSCs) possess, in a unique way, both the abilities of self-renewal and differentiation into many types of cancer cells. Because of their uncontrolled divisions and immortality, these types of cells are considered to be responsible for cancer development and metastasis as well as drug resistance [114,115,116,117,118]. Recently, it was demonstrated that coding RNAs (miRNAs) plays an important role in the regulation of some CSCs properties [53]. For these reasons, cancer stem cells are important for clinical and comparative oncological studies and, thus, one of the main areas of focus for the new anticancer therapy is to target cancer stem cells. Before establishing new targeted therapy on CSC, it is mandatory to characterize and isolate precisely this type of cells. This analysis is possible by using a panel of markers expressed by tumors of different histological types like: Sca-1 (stem cell antigen 1), CD24, CD44, CD133, CD166, EpCAM, and various integrins [119]. In canine mammary oncology, the role of cancer stem cells has been less investigated, but Magalhaes et al. (2013) found out that these cells express CD44, CD49f, Sox2, and Oct4 and most of the markers expressed are different from human CSC [120].

In general, cancer stem cells represent a small part of the tumor mass. In a study performed by Al-Hajj et al. (2003) on breast cancer cell lines, the population of CSC expressing ESA+/CD44+/CD24 was 2-4% [121]. Fillmore and Kuperwasser (2008) discovered that only a percentage of 0.02% to 0.5% CSC expressing ESA+/CD44+/CD24– cells was found in luminal subtype of breast cancer and 2.5% in basal-like subtype [122].

Rybicka et al. (2015) discovered 0.2–1.2% cancer stem like cells from the whole population of canine mammary neoplastic cell lines taken in study: CMT-U27, CMT-309 and P114 [123]. The results showing an increased colony formation units measured with colony formation assay (CFA) obtained by Rybicka et al. (2015) in CMT-U309, CMT-U27 and P114 cell lines were similar to those described by Fillmore and Kuperwasser (2008) in the subpopulation of human breast cancer stem cell lines SUM149 and SUM159 expressing CD44+/CD24–/ESA+. These CSCs can also can self-renew and reconstitute the differentiation spectrum of the parental cell line.

The role of miRNAs in CSCs development and maintenance

Concerning the role of miRNAs involved in breast cancer stem-like cells maintenance, there have been described so far only only let-7, miRNA-128 and miRNA-27a [124,125,126,127,128,129]. Yu et al. (2007) discovered that down-regulation of lethal-7 (let-7) molecules, a family of miRNAs involved in cell differentiation is important for self-renewal and mammosphere formation in breast CSCs [124]. In a study made by Zhu et al. (2011) miRNA-128 has been shown to be down-regulated in breast CSCs [125]. Tang et al. (2014) reported an increased expression of miRNA-27 and a reduced level in expression of its target gene ZBTB10 in breast cancer stem cells. Also the up-regulation of miRNA27a and the knockdown of ZBTB10 gene promoted tumourigenesis, angiogenesis and metastasis of breast cancer stem cells in NOD-SCID mice [125]. All these results were contrary to the results obtained by Rybicka et al. (2015) [123].

Recently one of the main interests in breast cancer research has been focused on the role of miRNAs in regulation of Transforming growth factor beta (TGF-β) signaling which is responsible for many pathological stages like cancer [130,131]. Wang et al. (2011) found that miRNA-181was involved in TGF-β signaling in breast cancer stem-like cells [132]. Rybicka et al. (2015) conducted a study to analyze the miRNA expression profiles in canine mammary cancerstem-like cells (expressing stem cell antigen 1, Sca-1; CD44 and EpCAM) isolated from canine mammary tumour cell lines CMT-U27, CMT-309 and P114. In this study it was shown that the main miRNAs implicated in TGF-β signaling are represented by: let-7 family, miRNA-27a, miRNA23a, miRNA-128, miRNA-106a and miRNA144. The qRT-PCR results revealed that the genes TGFBR1, TGFBR2, SOS1, CHUK, PDGFRA, MEF2A, MEF2C and MEF2D targeted by down-regulated miRNAs presented a

significantly higher expression in TGF-β signaling pathway in CSCs compared to the differentiated cancer cells from the same cell lines [123]. The discovery made in this study is in accordance with previous studies which have revealed the role of TGF-β in the maintenance of stem cells renewal, differentiation and thus might support tumorigenesis [133]. The results from human and canine studies highlight the importance of TGF-beta signaling pathway in breast cancer stem-cell biology and raise the possibility for a potential targeted therapy. Likewise, finding a set of miRNA markers for characterizing breast cancer stem cells and the possible use of miRNAs in targeted therapy for CSCs requires further investigation.

The role of the Wnt pathway in canine mammary cancers

The deregulation of the Wnt pathway in mammary cancer has been proven to be similar in both canines and humans [134].

The canonical Wnt signaling pathway, mediated through the β-catenin protein is activated when the Wnt ligands disrupt in the cytoplasm in the β-catenin complex, thus permitting the translocation of stabile β-catenin into the nucleus and to connect with T-cell factor (TCF)/Lymphoid enhancer binding factor (LEF)-family of transcription factors to regulate the expression of specific target genes.

Howe and Brown (2004) observed that the canonical Wnt signaling was expressed aberrantly in human breast tumors, while in studies made on human breast cancer cell lines there have been observed contradictory results [135].

In addition, a study performed on canine mammary carcinoma cell lines CMT1, CMT-U27 and CMT9 by Gracanin et al. (2014) also reported a highly active canonical Wnt signaling pathway, implicitly TCF-reporter activity was high and it was not sensitive to inhibitors of Wnt ligand secretion.

Generally, mutations occur in some key components of the Wnt cascade in cancer cell lines with a highly active canonical Wnt signaling pathway, but Gracanin et al. (2014) didn't find any mutations in the components from the coding regions of intracellular Wnt pathway (APC, β-catenin, GSK3b, CK1a and Axin1). The CMT1, CMT-U27 and CMT9 canine mammary cancer cell lines with high Wnt activity overexpressed in a notably way the LEF1 gene and the knock-down of this gene inhibited significantly the activity of TCF-reporter, therefore the Wnt activation could also occur in a ligand-independent way [136]. To conclude, in canine mammary tumor cells the Wnt activity can be activated both through moderate ligand-depent and high ligand-independent mechanisms.

Multiple drug resistance

One of the main cause responsible for chemotherapy failure is represented by multiple drug resistance which occurs mainly when a major membrane pump P-glycoprotein (P-gp) is overexpressed. The studies made by Lee et al. (1996) [137] and Pawłowski et al. (2013)

[138] shown that P-glycoprotein is responsible for the drug-resistant phenotype which can lead to chemotherapy failure. It has been demonstrated by Linn et al. (1995) that the expression of P-gp in tumoral cells has a prognostic value in patients with primary breast cancer and is considered to be a feasible biomarker for a more malignant and invasive phenotype [139]. Furthermore, recent studies made by Król et al. (2010) [140] and Pawłowski et al. (2013) [138] found a higher expression of P-gp invasive canine mammary cancer cell lines (CMT-W1, CMT-W2, CMT-U27, CMT-U9).

Król et al. (2014) treated with vinblastine two canine mammary tumors cell lines (CMT-W1 and CMT-W2) and two cell lines isolated from their lung metastases (CMT-W1M and CMT-W2M) in order to evaluate the effect of P-gp inhibitors verapamil and cyclosporine A and their possible implication in multidrug resistance. The results from qRT-PCR revealed that the multidrug-resistance gene (MDR1) known to be involved in P-gp synthesis was overexpressed in all canine mammary cell lines [141].

Tumorigenicity and chemoresistance of canine mammary cancer cell lines

In both species, human and canine, the ductal and lobular system from the mammary gland is composed of two major epithelial cell types: luminal and myoepithelial cells. A series of immunohistochemical studies characterized in both human and canine species the luminal cells by the expression of cytokeratins 7, 8, 18, 19 and epithelial membrane antigen, while most myoepithelial cells were characterized by the expression of vimentin, cytokeratin 14, cytokeratin 17, p63 and smooth muscle actin [37,142,143,144].

Chang et al. (2010) established and characterized three new canine mammary cancer cell lines: DE-E with epithelial morphology, DE-F with fibroblast morphology and DE-SF with spindle fibroblast morphology derived from a malignant tumor surgically removed from an 11-year-old mixed breed female dog. All of the canine mammary cancer cell lines DE-E, DE-F and DE-SF were positive for vimentin expression analyzed through immunocitochemistry and negative for cytokeratins 14, 17, 18, 19, p63, progesterone receptor, estrogen receptor, p53, Bax and Bcl-2 analyzed through western blot. Smooth muscle actin was only detected in DE-E and DE-F canine mammary cancer cell lines [57]. Under normal conditions, in healthy canine mammary the levels of estrogen and progesterone are relatively high and they decrease in case of malignancy [22]. Studies performed on canine mammary tumors by Sartin et al. (1992) [145] demonstrated that that the lack of expressing estrogen or progesterone receptors was correlated with a shorter overall survival, while Kesse-Adu and Shousha (2004) [146] followed by Sheik et al. (1994) [147] observed that the absence of estrogen receptors expression was associated with metastases. In human breast cancer, estrogen receptors expression

is present in about 70% of the cases and patients with a positive ER/PR status exhibit a better prognosis than those with a negative ER/PR status [148,149].

For more than two decades, it has been shown that the lack in expressing estrogen receptors in mammary tumors of both human and canine species was correlated with an increased chemoresistance to anti-estrogen drugs like tamoxifen [45]. The study made by Chang et al. (2010) on three ER negative canine mammary cancer cell lines (DE-E, DE-F and DE-SF) confirmed that chemoresistance to tamoxifen, melatonin, cyclosporine A and indole treatment varies among each cancer cell type. Only DE-F and DE-SF cell line developed tumorigenicity when injected into nude mice.

Concerning the resistance to chemotherapy, only DE-E cell line exhibited chemoresistance to doxorubicin. A recent study made by Chen et al. (2009) found that chemotherapy resistance was associated with a group of microRNAs expressed differentially in human breast cancer cell line MCF-7 [150]. Hence, the DE-E cell line may be used as a valuable in vitro model for comparative oncological studies in analyzing the potential role of miRNAs in chemoresistance in mammary tumors.

Given the fact that these canine mammary cancer cell lines present a differential tumorigenicity and chemoresistance, it is worth considering them as a suitable in vitro model for comparative oncological studies of mammary cancer carcinogenesis, chemoresistance and development of a potential anti-cancer therapy.

The role of RASSF1A tumor suppressor gene

Agathanggelou et al. (2005) observed that the gene RASSF1A also known as Ras association (RalGDS/AF-6) domain family member 1 was up-regulated in human cancers and presented tumor suppressor properties [151]. Krol et al. (2010) discovered that in both CMT-W1M and CMT-W2M cell lines isolated from lung metastases of canine mammary adenocarcinoma the expression of RASSF1A decreased in vitro colony formation and in vivo tumorigenicity [152]. This gene encodes a protein similar to the RAS effector of GTP-binding proteins involved in membrane remodeling by increasing cell adhesion, motility, and invasion thus inducing apoptosis and activating the metastatic process.

Breast cancer metastases

The final phase in the progression of breast cancer is represented by the process of metastasis, which usually occurs in bones and lungs, and less frequently in liver or brain.

Research studies have been carried out to identify a possible molecular signature for metastasis by comparing molecular portraits of primary tumors with their metastases. Fidler (1973) has made a hypothesis in which metastasis is determined by the activation of specific cellular pathways or genetic mutations in the evolution of the primary tumor

[153]. To strengthen this hypothesis, the studies performed by Weigelt et al. (2003) [154] and Kakiuchi et al. (2003) [155] it was revealed that the gene expression levels are different in each site of metastasis (lung, liver, kidney, bone).

The growth hormone secretagogue receptor (GHSR) also known as the ghrelin receptor might serve as an important biomarker in establishing a metastatic gene signature. The crucial role of growth hormone (GH) in breast carcinogenesis has been highlighted in some previous studies conducted by Mol et al. (1995) [156] on canine mammary tumors, Gil-Puig et al. (2002) [157] on MCF 7 adenocarcinoma cell line and Krol et al. (2009) [158] on simple canine carcinoma CMT-U27 and spindle-cell tumor CMT-U309 cell lines.

The growth hormone secretion is regulated by ghrelin an internal ligand to the GHSR. Ghrelin has been reported to be involved in breast cancer carcinogenesis by stimulating cell proliferation within an autocrine or paracrine mechanism. Variation in expression of ghrelin receptor was firstly reported in 2005 by Jeffrey et al. who observed that treatment with ghrelin significantly increased the proliferation of MDA-MB-435 and MDA-MB-231 breast cancer cell lines [159]. Krol et al. (2010) also found that GHSR was overexpressed in the CMT-W1M cell line isolated from lung metastases of canine mammary adenocarcinoma [160]. The mechanism regarding breast cancer proliferation induced by ghrelin still remains unknown, but it is presumed that it is activated by the stimulation of the mitogen-activated protein kinase pathway.

Forthcoming expectations

Nowadays, one of the most important achievements in human and veterinary oncology is the discovery of potential molecular biomarkers that could lead to earlier breast cancer detection, a more accurate characterization of breast cancer molecular mechanisms, followed by a successful development of effective therapeutic strategies in order to support a better outcome and to improve the overall survival of the patient.

Establishment of an experimental animal model for the study of human breast cancer will improve testing potential anti-cancer therapies which will lead to the development of personalized medicine or discovery of effective therapeutic schemes suitable for human clinical trials. Moreover, another important goal of experimental and clinical oncology is the development of efficient therapies to prevent cancer chemoresistance or multiple drug resistance.

The increased number of similarities between human and canine species presented in this review strengthens the proposition that it is worth considering the canine mammary cancer cell lines as a valuable in vitro model for breast cancer research.

Acknowledgements

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