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REVIEW ARTICLE

Novel promising serum biomarkers for canine mammary tumors

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Abstract
Canine mammary tumors (CMT) are the second most common form of neoplasia accounting for 40% of tumors in dogs. Today, as we know cancer is a disease of multiple genes and molecules. Research across the last three decades has enhanced our understanding of these molecules which is making possible new strategies of predicting, diagnosing and clinical treatment of this malady. Biomarkers are the modern day "neoplastic signatures" which enhances the specificity of tumor diagnosis allowing the researchers to differentiate tumors at the molecular level. This review enlists a concise summary of novel serum biomarkers counting matrix metalloproteinases, lysyl oxidase, heat shock proteins, mammaglobin, kynurenine 3-monooxygenase, WT1, nectin 4, and ERBB2. These new-fangled markers of canine malignancy could might help in early and accurate diagnosis resulting in more effective treatment and better prognosis. Nevertheless, more detailed research of these biomarkers along with their role in other cancers should be further validated in future.

Keywords Canine mammary tumors · Biomarkers · Canine · Human breast cancer · Veterinary oncology · Mammary glands

Abbreviations

BRCA1	Breast cancer 1 gene
BRCA2	Breast cancer 2 gene
CMT	Canine mammary tumors
COX-2	Cyclooxygenase
CRC	Colorectal cancer
ECM	Extracellular matrix
ERα	Estrogen receptor alpha
ERBB2	Erythroblastic leukemia viral oncogene homolog 2
GRP94	Glucose regulated protein 94
HCC	Hepatocellular carcinoma
HER-2	Human epidermal growth factor receptor 2
HIF-1α	Hypoxia-inducible factor 1α
HSF1	Heat shock factor 1
HSPs	Heat shock proteins
IHC	Immunohistochemistry
KMO	Kynurenine 3-monooxygenase
LOX	Lysyl oxidase
MAPK	Mitogen-activated protein kinase
MDCK	Madin darby canine kidney cells
MMPs	Matrix metalloproteinases
NMDA	N-methyl-D-aspartate
OSCC	Oral squamous cell carcinoma
PCNA	Proliferating cell nuclear antigen
qRT- PCR	Real time quantitative reverse transcriptase polymerase chain reaction
TGF- β1	Transforming growth factor beta1
TNF- α	Tumor necrosis factor—α
WT1	Wilm's tumor gene

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Introduction

The prevalence of cancer in dogs is a grave challenge that is frequently met by veterinarians and has increased owing to better nutrition, sophisticated diagnostics, vaccination, leash laws that limit automobile deaths and treatments for many life threatening ailments. Advances in the care of

animals have improved the general health of pets allowing them to live longer nevertheless resulting in an increase in age related diseases including cancer (Paoloni and Khanna 2008). Spontaneous tumors in companion dogs are very common and represent an important underutilized resource in cancer research. Mammary gland tumors, skin tumors, osteosarcomas and haemopoietic tumors are the most common malignancies encountered in dogs with mammary gland tumors being the second most frequent neoplasia in dogs, surpassed only by skin tumors (Todorova 2006). CMTs (Canine mammary tumors) are the most abundantly observed neoplasms in intact female dogs and accounts for 40% of tumors in these dogs (Pinho et al. 2012). Mammary neoplasia occurrence varies greatly in different species among which dog is the most frequently affected domestic species, with a prevalence of ~ 3 times that in human (Moe 2001). Mammary tumors are rare in cows, mares, goats, ewes, and sows. Fifty percent of older dogs develops cancer and it is estimated that one in four dogs eventually die from it (Bronson 1982). Incidence rate of 35–40% has been reported in India of which one third to half of them are malignant (Gupta et al. 2012).

Several causes of chemical, dietary, radiological, parasitic, mechanical and viral nature may have a role in etiology of canine mammary tumors. Other risk factors include age, heredity, hormones, and obesity (Lim et al. 2015). Certain breeds display an increased predisposition to cancer. Some breeds, e.g. poodle, spaniels, German shepherds, maltese, yorkshire terriers and dachshunds, seem to have an increased risk and purebred dogs are generally at a greater risk of developing CMTs and accounts for 80% of the submitted cases (Salas et al. 2015). In a study, breed most commonly affected from canine mammary tumors was samoyed/white spitz (34.10%) followed by doberman (19.65%), german shepherd (9.83%), non-descript (8.10%), labrador retriever (7.57%), pointer (5.78%), crossbred (5.20%), dachshund (2.89%), boxer (2.31%), cocker spaniel (1.73%), lhasa apso (1.16%), tibetian mastif, poodle and irish setter breeds (0.58% each) (Palta 2000). Many genes have been investigated in order to determine the extent to which genetic factors may predispose CMT. For instance, *p53* gene is involved in mammary tumorigenesis. The *p53* gene encodes a protein which normally regulates cell cycle and programmed cell death. In both benign and malignant types, *p53* mutations have been detected (Muto et al. 2000). Further, malignant tumors have been associated with absence of nuclear BRCA1 expression including high proliferation index and absence of ERα expression (Nieto et al. 2003).

Middle aged and older bitches are usually susceptible to mammary tumors. Although, an average age of occurrence from 8 to 10 years has been reported, a significant difference has been found in the onset of benign and malignant tumors; the benign tumors have a mean age of 8.5 years while the malignant tumors have a mean age of 9.5 years (Sorenmo et al. 2009). Mammary tumors are generally rare before 5 years of age (Schneider 1970). Early life exposure to ovarian hormones, mainly estrogens and progesterone, is known to play a key role in the development of CMTs. The risk for developing canine mammary tumors in dogs depends on the age at which the bitch is ovariectomized. Bitches spayed before puberty have 0.5% of risk, while those spayed after one estrous cycle have 8%, and those neutered after the second estrous cycle have 26% of risk (Schneider et al. 1969). An exogenous exposure to pharmacological doses of hormones such as estrogens and synthetic progestins used in veterinary practice has been shown to enhance tumor formation (Selman et al. 1994). Mammary adipose tissue contributes to increased exposure of estrogens which mediates mammary tumorigenesis (Simpson and Zhao 1996). In rodents, a strong correlation was observed between obesity and high occurrence of mammary tumors (Seilkop 1995). Further, homemade meals and red meat were found notably associated with a high incidence of tumors (Perez et al. 1998). Different methods for classification of CMTs have been proposed. However, 'International Histological Classification of Mammary Tumors of the Dog' by WHO is now generally accepted (Online Resource 1) (Misdorp et al. 1999).

The signs and symptoms of canine mammary tumors in dogs are not very clear, thus making the early stage diagnosis in dogs very difficult. A complete physical examination including careful palpation of the mammary glands along with thorough history may be helpful in determining diagnosis. In middle to older dogs, there are chances of suffering from other ailments as well. A mammary tumor is usually suspected on detection of a mass during physical examination. The length of time the mass has been present is usually unknown, but the rate of growth may be helpful in determining prognosis. Although the prevalence of these tumors decreases in regions where preventive ovariohysterectomy is performed, it still remains an important disease in veterinary medicine. By the time it is diagnosed, it has already reached an advanced stage and usually metastasized also. Further, treatment options in canine mammary tumors are very limited, therefore more emphasis should be on a timely diagnosis. Therefore in recent times, research is focussed on search of novel molecular markers or serological indicators like tumor specific or tumor associated antigens e.g. matrix metalloproteinases (MMPs), mammaglobins, heat shock proteins, extracellular matrix (ECM) remodelling enzymes such as lysyl oxidase (LOX) and many other antigens. These serum proteins might help in early and accurate diagnosis resulting in more effective treatment and better prognosis.

An integral part of patient care in this day and age would be curtailed without the laboratory diagnosis. The prospect of strong prognostic and/or predictive markers to identify patients at higher risk as well as selecting the most appropriate treatment is of utmost importance for clinicians both in

human and veterinary medicine. Tumor markers are usually proteins produced by tumors or other cells of the body in response to tumor or certain benign conditions. These proteins called as tumor associated antigens are abnormal in a tumorous patient when compared to a healthy one (Henry 2010) and their concentrations are much higher during cancerous conditions when measured in tissues, serum or urine. These markers play a crucial role in prognosis, diagnosis and early detection of metastasis. Therefore, emphasis on timely diagnosis to improve the prognosis with the help of biomarkers is need of the hour. Exploring novel serum proteins for early diagnosis may also play a major role in tumor research. Biomarkers of human breast cancer are also detectable in canine mammary tumors due to similar immuno-histochemical features between the two (Pena et al. 2014). The choice of the most appropriate biomarker is still a major challenge amidst the heterogenous biology of mammary tumors.

Biological markers in canine tumors

So far, based on the current literature miRNA, cancer stem cells, circulating tumor cells, breast cancer 1 gene (BRCA1), breast cancer 2 gene (BRCA2), Ki-67, endothelial growth factor receptor, human epidermal growth factor receptor 2 (HER-2), estrogen receptor, progesterone receptor and cyclooxygenase (COX-2) come under the most promising biomarkers (Kaszak et al. 2018).This review will describe additional novel serum biomarkers which have both prognostic as well as diagnostic potential in CMTs. Conversely, additional studies on a more homogenous group and higher number of samples must be carried out for their validation.

Matrix metalloproteinases

MMPs constitute a family of extracellular zinc and calcium—dependent proteinases mediating a vital role in multiple biological processes such as cell growth, invasion, angiogenesis and metastasis (Chang and Werb 2001). MMPs have four structural domains; N-terminal signal peptide, the propeptide, the catalytic domain, and the hemopexin-like C-terminal domain linked to the catalytic domain by a flexible hinge region (Verma and Hansch 2007). They are also known to hydrolyze the extracellular matrix (ECM) and so the name matrixins. The MMP family is classified into 26 subgroups based on their substrate specificity and structure: Gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-12), colleagenases (MMP-1, MMP-8, MMP-13, MMP-18), matrilysins (MMP-7, MMP-26), membrane type MMPs (MMP-14, MMP-15, MMP-16, MMP-17) and others (MMP-11, MMP-19, MMP-20) (Somerville et al. 2000). MMPs help each other to create a surge of activation wherein an activated MMP, turns on the fellow MMPs and as a consequence, degrades the ECM. These enzymes also help to invade the cancer cells by acting as molecular scissors to cut through the proteins, hence restricting the movement of cancer cells. Once the cancer cells have traversed the basal lamina, they can either enter the bloodstream or re-enter the tissues forming tumor at newer locations, consequently metastasizing tumors (Yoshida et al. 2007). Elevated expression of MMPs can be studied in conditions like cancer, thus enabling the researchers to justify their metastatic role.

MMP-3

MMP-3 (Sromelysin-1) synthesized by stromal fibroblasts, synovial cells and macrophages (Tetlow et al. 1995) has been found to have a proteolytic action on ECM components. It is also crucial for the generation of fully active MMP-1 and MMP-9 from their partially processed pro-MMPs (Suzuki et al. 1990). MMP-3 induces apoptosis in differentiated mammary alveolar epithelium along with proliferation and branching of ductal epithelium, thus an altered stromal environment potentially endorsing metastasis in breast cancer (Alexander et al. 1996). In humans, high expression of MMP-3 in tumor tissues is correlated with a distorted stromal setting, angiogenesis, cell proliferation, and apoptosis possibly promoting breast cancer. MMP-3 over expression is also reported in majority of human carcinomas including prostate, liver, bone and the squamous epithelium (Nelson et al. 2000). So far, there is only one study in canines linking invariable up regulation of MMP-3 to CMTs. MMP-3 expression and its detection in body fluids may serve as a marker for diagnosis of tumors in canines (Pandey et al. 2017). A canine specific ELISA kit evaluated the role of MMP-3 as a serum biomarker (Pandey et al. 2017). The serum levels of MMP-3 correlated positively with tumor grade differentiating healthy dogs from the tumor-bearing dogs. MMP-3 seems to be a sensitive, non invasive and a promising biomarker for sero-diagnosis of canine mammary carcinomas.

MMP-11

MMP-11 (Stromelysin 3) has been detected in immediate vicinity of cancer cells, stromal cells as well as adjacent microenvironment which promotes cancer growth by inhibiting apoptosis as well as increased migration and invasion of cancer cells (Zhang et al. 2016). In humans, MMP-11 was found to be aberrantly expressed in fibroblastic cells of invasive breast carcinomas (Rouyer et al. 1994) and other human carcinomas (Basset et al. 1990). Expression of MMP-11 was also greatly predictive for the occurrence of distant metastasis. High expression levels of MMP-11 mRNA or protein is correlated with a poor prognosis in human breast

cancers (Engel et al. 1994). It has been targeted as a novel antigen in cancer immunotherapy (Peruzzi et al. 2009). Evidence of involvement of MMP-11 in tumorigenesis has been well acknowledged in vivo. In veterinary medicine, elevated expression of MMP-11 has already been revealed during mammary tumor progression in dogs (Klopfleisch et al. 2011, Sunil Kumar et al. 2012). The study reports the gene expression profile of MMP-11 in dogs with mammary tumors by real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). An upregulation of MMP-11 (4.323 times) was reported in mammary tumor subjects when compared with the healthy subjects. In another study in dogs, rMMP-11 mature peptide was found to degrade the ECM and endorsed invasiveness to MDCK cell line suggesting the potential role of MMP-11 in tumor progression (Sunil Kumar et al. 2013). The ability of the tumor cells to modulate the surrounding ECM is a quintessential for invasion to happen wherein the MMP-11 plays the role of ECM modulator proteases. These experimental evidences have been overwhelming in proving that MMP-11 has a great potential to be a new mammary tumor marker. However in both human as well as canines, the role of MMP-11 should be evaluated together with MMP biomarkers such as MMP-3.

Lysyl oxidase

LOX is an extracellular matrix enzyme mediating the crosslinking of collagen and elastin (Mäki 2009). Expression of LOX is highly responsive to a variety of physiological states such as growth, wound repair, ageing, genetic diseases involving altered copper metabolism and tumorigenesis. Aberrant activation or expression of LOX often leads to diseases like tissue fibrosis and cancer (Barker et al. 2012). Expression of LOX can be regulated at the transcriptional or post translational level. Apart from that, other factors including cytokines and growth factors have also been found to be involved in the regulation of its expression. One of the key cytokines involved in regulating the ECM, TGF- β1 promotes LOX mRNA expression level in a dose and time dependent manner via the activation of Smad3, PI-3 kinase and mitogen-activated protein kinase (MAPK) signaling (Taylor et al. 2011). TNF- α, a pro-inflammatory cytokine drives its expression during chronic inflammation via the reactive oxygen species-activated NF-kB/extracellular signal-related kinase pathway. This promotes progression of breast cancer metastasis and cardiac fibrosis (Voloshenyuk et al. 2011). Hypoxia-inducible factor 1α (HIF-1α) has also been identified in regulating its expression by enhancing cell–matrix adhesion, migration, invasion and metastasis (Erler et al. 2009). Hypoxia also induces stromal expression of LOX causing collagen linearization, increasing ECM stiffness and induces epithelial loss in cancer cells, thus enhancing tumor cell invasion through ECM remodeling (Mayorca and Erler 2013).In humans, many studies have done to study role of LOX in cancers. High LOX expression induces metastasis in various cancers such as oral squamous cell carcinoma (OSCC) (Albinger et al. 2010), colorectal cancer (CRC) (Baker et al. 2011), breast cancer (Bondareva et al. 2009) and lung adenocarcinoma (Wilgus et al. 2011). Taylor et al.,(2011) in their study showed TGF-β1 stimulated the synthesis and secretion of LOX from normal and malignant MECs in vitro and in mammary tumors produced in mice. Their study identified LOX as a potential mediator that couples mechanotransduction to oncogenic signaling by TGF-β1 and suggested breast cancer progression stimulated by TGF- β1 is diminished by inactivating LOX. In veterinary medicine, only one study has been conducted to evaluate the role of LOX as a serum biomarker, using a canine specific ELISA kit for its determination (Saleem et al. 2019). The serum levels of LOX correlated positively with tumor grade differentiating healthy dogs from the tumor bearing dogs. The study also reported a significant (threefold) upregulation of LOX gene in mammary tumor cases as compared to healthy dogs suggesting its possible role as a sensitive and non invasive tumor biomarker in CMT. In both humans and canines, LOX overexpression correlates with an unfavorable prognosis. Nevertheless, it is also believed LOX probably exerts a synergistic effect along with MMP-2 and MMP-9 on ECM remodeling and tumor cell invasion (Liu et al. 2012). Thus, it is recommended to evaluate the expression of LOX in combination with other biomarkers especially MMPs. In veterinary medicine, studies reported were based on a small number of heterogeneous samples. Therefore, more studies need to be done to validate its role as a biomarker.

Heat shock proteins

HSPs are described as regulators of hyperthermia resistance in all cellular organisms (Calderwood and Ciocca 2008). They are further characterized as members of the molecular chaperones, a group of proteins that play essential roles in the correct folding of cellular proteins (Bukau et al. 2006). In addition, HSPs participate directly in inhibiting programmed cell death and cell senescence. At least two members of the HSP family, HSP27 and HSP70 undergo significant increases in cellular concentration and block the programmed cell death and replicative senescence pathways that often accompanies malignant transformation. This results in HSP-mediated inhibition of tumor cell inactivation (Calderwood 2010).

Hsp27

Activation of Hsp27 in stress takes place both by transcriptional activation and post-translational modification

(phosphorylation) acting downstream of the p38 MAPK stress kinase pathway (Brunet et al. 2009). Heat shock factor 1 (HSF1) has been reported to play a function in breast cancer progression by inducing Hsps. In humans, aberrant expression of Hsp27 has been correlated with the invasiveness of breast cancer cells furthermore; their metastatic potential has been well demonstrated (Lemieux et al. 1997). In breast cancer, HSP27 exerts pro-malignant effects largely due to its properties of inhibiting programmed cell death and senescence (Sherman et al. 2007). In veterinary medicine, there have been a few studies confirming that Hsp27 is aberrantly expressed in CMT as well (Bongiovanni et al. 2014; Birdi et al. 2019). Using IHC, Hsp27 was found to be overexpressed in canine mammary tumors (Bongiovanni et al. 2015). Additionally, it has been reported that circulating levels of Hsp27 is elevated in dogs with mammary tumors. Hsp27 has been found to have a positive correlation with tumor grade (dogs with grade-II tumors had higher Hsp27 levels as compared to grade-I types) (Birdi et al. 2019). In both human and animal medicine, Hsp27 seems to be a good serum biomarker for both prognostic and therapeutic significance in breast cancer and CMTs.

Hsp90

Hsp90 is one of the most abundant proteins in the mammalian cells which are classified into four major isoforms: TRAP1 (mitochondrial form), Hsp90A (cytosolic form)—HSP90AA.

(inducible), HSP90AB (constitutively expressed) and Hsp90B1(ER form/Grp94). Hsp90 chaperones are aberrantly expressed and mutated oncoproteins and in doing so, stimulate the tumor progression (Cheng et al. 2012). Tumorigenesis induced nutrient and hypoxic stress damages the cell's protein machinery and results in building up of damaged proteins in the endoplasmic reticulum. In order to maintain the cell homeostasis, glucose regulated protein 94 (Hsp90b1) expression elevates in the endoplasmic reticulum. Grp94 is also associated with cancer cell invasion and proliferation (Nami et al. 2016). In humans, abnormal levels of Hsp90B1 has been reported in breast cancer cells with distant metastasis and decreased overall survival (Lee 2014). Poor prognosis, increased likelihood of recurrence and more aggressive breast cancer are often allied to overexpression of Hsp90 which signals the cells to grow continuously and develop malignant tumors (Cheng et al. 2012). In a recent study in dogs with mammary tumors, serum concentrations of Hsp90B1/Grp94 protein were efficient enough to differentiate CMT subjects from the healthy patients suggesting its use as a serological biomarker as well as to evaluate the success of targeted therapy. Elevated expression of Hsp90B1 mRNA and protein were reported to be associated with mammary carcinogenesis (Kumar et al. 2017). Hsp90B1 has been demonstrated as a valuable cancer diagnostic marker owing to it immune-modulating activities on immune cells.

Mammaglobin

A glycoprotein by nature, mammaglobin exhibits similarity to secretoglobin-uteroglobin family. It has been reported to act as a diagnostic marker for breast carcinoma (Wang et al. 2009), though the exact function of the protein is not clear. Independent of the stage and grade of tumor, mammaglobin expression was found elevated in breast cancer patients. Further, aberrant expression of mRNA was also depicted in 70–80% of primary and breast tumor biopsies (Leygue et al. 1999). In veterinary medicine, the use of mammaglobin for CMT detection has also been validated. Mammaglobin protein is only secreted in the blood of CMT patients (not in any other disease condition). A canine specific sandwich ELISA kit efficiently differentiated CMT subjects from the diseased ones and has been demonstrated as a novel, specific and sensitive technique (Pandey et al. 2018).This strongly indicates that mammaglobin is a mammary/breast tumor specific marker and is specific enough for detection of tumor subjects.

Kynurenine 3-monooxygenase

Kynurenine 3-monooxygenase (KMO), a key enzyme in the kynurenine pathway catalyzes the hydroxylation of L-kynurenine (L-Kyn) to form 3-hydroxy-L-kynurenine and generates the metabolite quinolinic acid (neurotoxic NMDA receptor antagonist) (Breton et al. 2000). Quinolinic acid may also affect NMDA receptor signaling in myocardial cells, pancreatic beta cells, gastrointestinal tract and the osteoblasts (Robert et al. 2012). NMDA receptors were reported to be highly expressed in human breast cancer cell lines (North et al. 2010). 3-hydroxyanthranilic acid, a metabolite of kynurenine induces apoptosis of Th1 cells and T-cells (Fallarino et al. 2002). KMO inhibitors have a potential role in neurodegenerative diseases therapy, as they balance receptor agonists and antagonists (Thevandavakkam et al. 2010). Currently, not much is known about its role in tumor development. However, KMO overexpression is associated with an aggressive malignant phenotype of human hepatocellular carcinoma (HCC), and poor overall survival (Jin et al. 2015). In veterinary medicine, cancer malignancy has also been correlated with overexpression of KMO and predicts a poor prognosis in CMT (Chiu et al. 2019). Further, both protein and gene expression of KMO was appreciably higher in stage IV and V tumors with an overall shorter survival time in CMT subjects. KMO could efficiently discriminate benign from malignant CMTs and around 73.7% of the malignant CMTs showed an aberrant expression of

KMO (Chiu et al. 2019). KMO as a tumor biomarker has potential applications in tumor prognosis and therapeutics.

WT1

WT1 (Wilm's tumor gene), a transcriptional factor regulates the expression of multiple genes implicated in cell proliferation, apoptosis, sexual differentiation (Hewitt et al. 1995). This gene is being extensively studied in humans as its elevated expression has been reported in various solid tumors like breast cancer, mesotheliomas, lung cancer as well as in leukemia (Xu et al. 2013). Further, the presence of WT1 in diverse solid tumors has also been confirmed by IHC (Nakatsuka et al. 2006). Current reports on WT1 expression analysis on human breast cancer confirmed presence of WT1 in neoplastic cells, specifically carcinomas associated with a poor prognosis (Miyoshi et al. 2002). In veterinary medicine, a similar study has reported the potential role of WT1 in CMT and described a positive correlation between CMT and WT1 expression using IHC and RT-PCR (Carranza-Martínez et al. 2019). Using IHC, WT1 expression was found to be positive only in those biopsies which were malignant suggesting it as a potential biomarker and an indicator of malignancy. However, further studies are required to elucidate the WT1 signalling pathways in the mammary tumors.

Nectin-4

Nectins are type I transmembrane glycoproteins and form adherens junctions in epithelial cells (Takai et al. 2008). They belong to immunoglobulin family with four members, Nectin-1, -2,-3 and -4 (Morrison and Racaniello 1992). Nectin-4 expresses itself during embryogenesis while the others express themselves during adult tissues. Nectin-4 normally lacks expression in a healthy mammary gland and its elevated expression has been reported both in human breast tumors and tumor cell lines (Fabre-Lafay et al. 2007). It might be a potential target as a tumor associated biomarker for human lung and mammary cancers as it has been shown to play a pivotal role in cancer cell growth and invasion (Takano et al. 2009). In veterinary medicine, there are only a limited studies of Nectin-4 in clinical and tumor biology cases. A comprehensive study of Nectin-4 together with PCNA and Ki-67 expression indicated a positive, linear and a high correlation in canine mammary carcinomas (Dolu and Aydogan 2018). IHC revealed the Nectin-4 expression around the plasma membrane and cytoplasm of the malignant tumor cells and its absence from the non cancer areas of the section. This suggests the use of Nectin-4 as a tumor associated biomarker in prognosis and diagnosis of mammary carcinomas. However, further studies are needed to evaluate the role of Nectin-4 along with other prognostic markers in canine mammary carcinomas.

ERBB2

Erythroblastic Leukemia Viral Oncogene Homolog 2 (ERBB2) is closely related to epidermal growth factor receptor (EGFR) and is involved in the signal transduction pathways leading to cell differentiation and growth (Akiyama et al. 1986). Super family consists of four members: ERBB1, ERBB2, ERBB3, ERBB4 which have an extracellular ligand binding region, cytoplasmic tyrosine kinase domain and a single membrane spanning region (Lupu et al. 1995). The ERBB receptors have been implicated during development and in normal adult physiology and are expressed in various tissues of neuronal, epithelial and mesenchymal origins. Elevated ERBB2 protein signals the cells to grow continuously, contributing to malignancy (Bertucci et al. 2004). In human breast cancer, ERBB2 mRNA was found at elevated levels and associated with an aggressive breast cancer (Bernstein et al. 2005). Over expression of ERBB2 has also been reported in malignant mammary tumors of pets (Klopfleisch et al. 2011). Expression profiling of ERBB2 gene has also been studied in dogs with mammary tumors and is associated with overall poor prognosis and inversely correlates with patient survival time (Chaudhary et al. 2015). More detailed study regarding its signal transduction pathway and its role in CMT should be done.

Conclusion

Over the past few decades, many studies have been carried out both in humans as well as in animals relating to mammary tumor biomarkers. However, no productive conclusions have been drawn out so far. The most promising biomarker should give a clear prognosis without any bias of other degenerative disease, should be tumor-specific and easily measured either in blood or tissues. Serum biomarkers are much more promising over tissue biomarkers as the dynamic physiological and pathological states are evident even before the clinical signs start appearing.There is a strong need to identify highly sensitive, accurate and reliable biomarkers for CMT diagnosis and therapeutics. Several examples of human breast cancer biomarkers have been proven to be detectable in CMTs emphasizing the challenges but also the opportunities at each step of biomarker development. However, there is still a large gap between the biomarker discovery and its successful clinical translation.

Biomarkers are considered invaluable tools for cancer detection, diagnosis and prognosis. Since malignancies have a very complex nature, to unravel the mystery of cancer diagnosis, and to obtain more reliable results effort

should be to detect multiple biomarkers instead of relying on just a single one. Further, cancer should be featured as a multistep process i.e., it doesn't develop all at once, and rather it is a complex succession of genetic changes over time. Multiple genes either oncogenes or tumor suppressor genes have a major role in triggering cancer. A key area of research lies in the exploitation of diverse cancer biomarkers (stage specific or cell cycle specific) which can be either diagnostic or may suggest new targets for anticancer therapies. Canines are an excellent experimental model for study of human diseases including breast cancer owing to the high homology in the genome sequences when compared to the human counterpart. With the advent of immunotherapy and immune-prevention strategy, dogs have been tested for the modalities in a pre-clinical settings as well as to explore the prevention strategies.

The biomarkers discussed above can be used to constitute a set of mammary biomarkers based on their link with tumor development. Nonetheless, most of the above discussed biomarker studies have been done on more heterogeneous samples or either on less number of samples. Detailed studies of these biomarkers in future will be required as these are interesting targets for further investigations and therapy both in human as well as in canine subjects.

Author contributions AS made substantial contributions to conception, design and acquisition of data and major interpretation of data. MG and AZ have been involved in drafting the manuscript and revising it critically for important intellectual content. All the authors have given their final approval of the version to be published.

Declarations

Conflict of interest The authors report no conflict of interest.

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