Jurnal Medik Veteriner
DOI: 10.20473/jmv.vol6.iss2.2023.271–287
October 2023, Vol.6 No.2, 271–287
online at https://e-journal.unair.ac.id/JMV

Mammary Tumors in Dogs, Recent Perspectives and Antiangiogenesis as a Therapeutic Strategy: Literature Study

Palagan Senopati Sewoyo[1], Anak Agung Ayu Mirah Adi[2]*, Ida Bagus Oka Winaya[2], I Wayan Wirata[3]

[1]Master Program, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia, [2]Department of Pathobiology, Laboratory of Veterinary Pathology, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia, [3]Department of Clinical, Laboratory of Veterinary Surgery, Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia.
*Corresponding author: aaa_mirahadi@unud.ac.id

Abstract

A particular type of tumor that is frequently detected in female dogs who are sexually active is a mammary tumor. Neoplasia results from DNA-based alterations in cell cycle regulating genes. The mammary gland is prone to the formation of tumors due to its dynamic structure. The development of this tumor is supported by numerous variables. It has been recently discovered that there is substantial evidence linking the BRCA2 gene to the process of cancer. Standard examination techniques, such as fine needle aspiration, histopathology, and immunohistochemistry, are used along with ancillary tests to determine the tumor type and degree of malignancy. The primary treatment option for malignant tumors is surgical resection followed by adjuvant chemotherapy; benign tumors necessitate surgical resection as well. Adjuvant therapy options include hormone therapy and non-steroidal anti-inflammatory medications. Tumor tissue undergoes angiogenesis as it grows and develops to accommodate the abundant supply of nutrients. Therefore, angiogenesis-inhibiting therapies can be utilized to halt the growth of tumor cells. A number of antiangiogenic medications are now being studied in clinical settings on humans, and several more are undergoing trials on animals. In addition to pharmaceuticals, viruses may be used as a therapeutic to block tumor angiogenesis.

Keywords: angiogenesis, dogs, mammary tumors, therapeutic targets

Received: 28 March 2023 **Revised:** 21 May 2023 **Accepted:** 23 June 2023

INTRODUCTION

One of the leading causes of death for companion dogs worldwide is malignant tumors. Although the exact prevalence of this illness is unknown in Indonesia, veterinarians frequently treat patients for it. Of all the tumor samples admitted to the Department of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University in 2019, the incidence of mammary gland cancers was 22,7% (Widyarini *et al*., 2022). Tumor initiation and progression are influenced by various factors such as age, gender, nutritional status, reproductive status, and exposure to environmental stimuli (Kaszak *et al*., 2022). Mammary tumors are common in sexually active female dogs. Certain breeds are more prone than others to acquire this type of breast tumor. This implies that congenital or genetic reasons may be involved. Until macroscopic abnormalities are noticed or the tumor is discovered during a routine clinical examination at the clinic, dog owners usually are unaware that their pet has a tumor. Around 25–70% of all tumor forms that affect female dogs worldwide are mammary cancers (Torres *et al*., 2021).

Mammary tumors can occur spontaneously in dogs. It is similar to human breast tumors in terms of clinical features, epidemiological patterns, and prognosis (Gray *et al*., 2020; Vascellari *et al*., 2016). The environment that both humans and dogs live in is becoming more contaminated with substances known as carcinogens, which can cause malignancies (Gray *et al*., 2020; Meuten, 2016). Currently, the most successful treatment is surgically excising the afflicted mammary gland together with the surrounding lymph nodes. However, surgery is not always curative in malignant cases with a high rate of metastasis (Sorenmo *et al*., 2020). Therefore, in some situations, adjuvant therapy

such as chemotherapy or radiation therapy may be used.

During the growth phase, tumors require a plentiful supply of nutrients to expand in size. Tumor cell proliferation requires the quick and vast formation of new blood vessels since these arteries serve as the pathways for this nutritional intake. As per the findings of Dileepkumar *et al*. (2015) and Katayama *et al*. (2019), the tumor's growth will not exceed 1–2 mm^3 in the absence of new blood vessel formation. Angiogenesis is the main target of the development of malignant tumor therapy in humans (Li *et al*., 2015).

Along with a fundamental overview of carcinogenesis, this literature study will explore into additional detail on the most recent findings regarding canine mammary cancers. In addition, this literature study addresses the mechanisms of angiogenesis and the genes involved, which may be blocked to provide therapeutic benefits, in light of the significant role angiogenesis plays in the development of tumors.

MATERIALS AND METHODS

This literature study article was written by collecting and accessing several scientific articles searched through Google Scholar, ScienceDirect, PubMed, and Research Gate sites. Keywords in the search were as follows: tumorigenesis, canine mammary tumor, angiogenesis mechanism, CMT therapy, and antiangiogenic drugs in veterinary. The inclusion criteria for this literature study were to include references in the form of books or journals within the last 10 years. The exclusion criteria were references older than 10 years. However, journals with these criteria can be included if they are considered the most up-to-date and there are no updates or new primary sources. The data obtained was compiled, analyzed, and concluded.

RESULTS AND DISCUSSION

Cell Cycle and General Study of Tumor

Cells are the smallest structural and functional units of living things (Ellinger and Ellinger, 2013). Metabolism is a chemical reaction that mostly occurs in cells, with the aim of enabling living things to carry out their activities. Each cell originates from the division of a previous cell (Figure 1). The process of DNA replication and cell division can be described as coordinated stages called the cell division cycle (Liu *et al*., 2022; Matthew *et al*., 2022).

Cell division through the cell cycle process is useful for regenerating damaged or aging cells. In addition, cell division is also needed to repair damage caused by injury or inflammatory processes (Canaud and Bonventre, 2015). In the main terms, the cell cycle is divided into two stages, namely interphase and mitosis (M). Interphase is divided into Growth phase 1 (G1), synthesis phase (S), and Growth phase 2 (G2) (Figure 1). Meanwhile, the resting phase is characterized by the loss of division activity, called the G0 phase (Liu *et al*., 2022; Matthew *et al*., 2022).

In response to DNA damage during interphase, replication stress during the S phase, or incomplete spindle assembly during the M phase, specific cell cycle checkpoints arrest or slow the cell cycle by inhibiting cyclin-dependent kinase (CDK) and anaphase-promoting complex/cyclosome (APC/C). The DNA damage checkpoint can be activated through double-strand DNA breaks (DSB) in interphase (Figure 2). Cell cycle checkpoints are critical in preventing the accumulation and cyclopropagation of genetic errors during cell division (Matthew *et al*., 2022).

DNA repair pathways, cell cycle arrest checkpoints, and cell death induction exist in cells with the aim of processing damaged DNA and preventing genome instability. Mutated DNA repair genes will result in a decrease in an individual's ability to repair DNA, thereby causing increased susceptibility to tumorigenesis. DNA damage can involve single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA) from DNA molecules. This damage is called single-strand DNA break (SDB) and double-strand DNA break (DSB) (Sadeghi *et al*., 2020).

DSB is very serious and dangerous due to it can cause extreme mutations. Several cellular activities such as DNA repair pathways, cell cycle

arrest, and apoptosis are important for repairing DNA damage, which aims to prevent uncontrolled cell division and pass on damaged DNA to daughter cells. Animals that have gene mutations in these pathways are more sensitive to radiation exposure (radiosensitive) and have proliferation abnormalities. This causes an increased risk of cancer (Sadeghi *et al*., 2020).

Changes that occur in cell cycle regulatory genes at the DNA level, especially in cell cycle checkpoint genes and DNA repair pathways, will cause neoplasia. Neoplasia is a process of new growth in cells that experience irreversible genetic changes. These transformed cells are unresponsive to normal controls and completely bypass existing cell cycle regulation. Proliferation occurs continuously until it exceeds normal anatomical limits, causing changes at the macroscopic and microscopic levels. Tumor development is a gradual process, starting with preneoplastic changes (Newkirk *et al*., 2017).

Preneoplastic changes can include hyperplasia, hypertrophy, metaplasia, and dysplasia. These changes signal an increased risk of neoplasia in this tissue. In general, preneoplastic changes are reversible. The appearance of these changes can be caused by a physiological response, injury, or irritation. For example, epidermal hyperplasia is a normal occurrence in the wound-healing process. Neoplasms develop as a result of genetic and epigenetic damage that occurs over a long duration (Figure 3). This accumulative effect will form a tumor. Tumor development is called multistage carcinogenesis or stepwise tumor development (Newkirk *et al*., 2017).

The first stage of carcinogenesis is initiation. At this stage, irreversible genetic changes occur in normal cells due to exposure to mutagenic initiating agents or initiators. Initiators are chemical or physical carcinogenic compounds that can damage DNA. Initiated cells appear morphologically normal and remain in a quiescent state for years. Even in a quiescent state, these cells are very sensitive to mitotic signals and more resistant to apoptotic signals than other normal cells (Newkirk *et al*., 2017). In cells that have been initiated, damaged genes cannot be repaired through DNA repair mechanisms or the apoptosis process (Astawa, 2018).

The second stage of tumor development is promotion. This stage is associated with an increase in the proliferative power of cells that have been initiated (Astawa, 2018). This stage will stimulate cells that have been initiated with a stimulus from a promoter agent. Promoter agents are generally not mutagenic and can create a favorable environment for proliferation. Because it is not mutagenic, the changes caused by promoter agents are reversible. The response to proliferative signals from promoter substances will build a larger population of initiated cells, thereby increasing the risk of further mutations occurring. At the end of the promotion stage, the result of this stage is a benign tumor (Newkirk *et al*., 2017).

In the progression stage, the final stage of tumor development, benign tumors will turn into malignant tumors in a process called 'malignant transformation' (Newkirk *et al*., 2017). Progression is associated with the occurrence of several additional mutations in one or several genes which cause tumor cells to proliferate more rapidly and invasively (Astawa, 2018). Hanahan (2022) summarizes the characteristics of malignancy into several criteria, including: (1) having the ability to stimulate its own growth by maintaining proliferative signals, (2) ignoring signals from oncogenes and tumor suppressors to slow growth, (3) resistance to programmed cell death (apoptosis), (4) stimulates angiogenesis, (5) carries out invasion and metastasis, (6) has immortal replication capabilities (works against telomere limitation), (7) reprograms energy metabolism, (8) avoids destruction of the immune system, (9) genome instability and mutations, and (10) increased tumor-mediated inflammation.

Mammary Tumors in Dogs

The mammary gland in dogs is a unique organ, which undergoes remodeling and differentiation during the life of reproductively active female dogs (Allard, 2014). In each estrous cycle, waves of epithelial and mesenchymal proliferation, ductal branching, and alveoligenesis are accompanied by intensive

regressive changes. All of this is coordinated with the hormonal levels of estrogen and progesterone (Michishita, 2020). Unfortunately, these physiological and morphological changes in the mammary glands are highly susceptible to neoplastic changes (Figure 4). In fact, mammary tumors are a common occurrence in female dogs (Kwon *et al*., 2023).

Globally, mammary tumors account for around 25–70% of the total types of tumors affecting female dogs (Torres *et al*., 2021). The development of mammary tumors is rare in dogs with < 5 years of age. In most cases, only benign tumors are diagnosed at this age. There are differences between races regarding the risk of developing mammary tumors, indicating a genetic influence on mammary gland tumorigenesis (Sorenmo *et al*., 2020). Currently, there is little evidence that mammary gland tumors have the potential for genetic mutations. From a microscopic perspective, mammary cancer is highly heterogeneous. This has led to improvements in the classification system for mammary tumors (Goldschmidt *et al*., 2011).

In recent years, the World Health Organization (WHO) has widely adopted veterinary pathology diagnostics. Mammary gland tumors can consist of luminal epithelial cells (simple tumors), or be associated with myoepithelial cells (complex tumors) and mesenchymal cells, such as cartilage or bone tumors (mixed tumors) (Misdorp, 2002). Dog breeds such as Dachshund, Cavalier King Charles Spaniel, Papillon, Pomeranian, Yorkshire terrier, and Maltese are reported to have a high incidence of mammary tumors (Komazawa *et al*., 2016). Mammary tumors are also observed more frequently in purebred dogs (62%) than in mixed breeds (38%) (Vascellari *et al*., 2016).

The Role of Gonad Hormones in Tumorigenesis

The appearance of mammary tumors is closely related to age and hormonal imbalance e.g., estradiol-17β, progesterone, and prolactin. The role of estrogen in influencing mammary gland carcinogenesis is very large (Canadas-Sousa *et al*., 2019). Exposure to this hormone in the first to second year is known to be a risk factor in developing this tumor (Kristiansen *et al*., 2016). Estrogen can activate other hormones such as relaxin which triggers further cell proliferation. Dominantly, estrogen plays a greater role than progesterone in the development of malignant mammary tumors (Torres *et al*., 2021).

Estrogen acts as a promoter of cells that have been initiated, so to minimize the development of mammary tumors it is recommended to perform an ovariohysterectomy to minimize the risk of its occurrence. In fact, it is recommended to perform an ovariohysterectomy at a young age, especially before the first estrus occurs (Torres *et al*., 2021). Ovariohysterectomy in old age does not reduce the risk of developing mammary tumors (Sorenmo *et al*., 2020), but increases the life expectancy of post-operative mammary tumor patients (Kristiansen *et al*., 2016). The incidence rate of mammary tumors in Europe in dogs is extremely high because ovariohysterectomy procedures are not routinely performed (Sorenmo *et al*., 2020).

Involvement of the BRCA2 Gene in Tumor

Breast Cancer Gene 2 (BRCA2) was first identified in 1995 (Wooster *et al*., 1995). This large gene contains 27 exons. In humans, mutations commonly occur in exons 10 and 11, either in the form of insertions or deletions, resulting in premature stop codons (Sadeghi *et al*., 2020).

The BRCA2 gene participates in various biological activities. This protein acts as a tumor suppressor gene and prevents cells from dividing uncontrollably by regulating DNA repair pathways, the cell cycle, and cell death (Sadeghi *et al*., 2020). In humans, mutations in the BRCA2 gene increase the risk of breast cancer by 45–85% and ovarian cancer by 11–23% in the female population (Enginler *et al*., 2014; Thumser-Henner *et al*., 2020).

Similar to BRCA1, BRCA2 plays an important role in the DNA repair system. Rats that are deficient in the BRCA2 gene show an unstable genome, their cells are more sensitive to DNA-damaging agents and fail to repair DNA damage

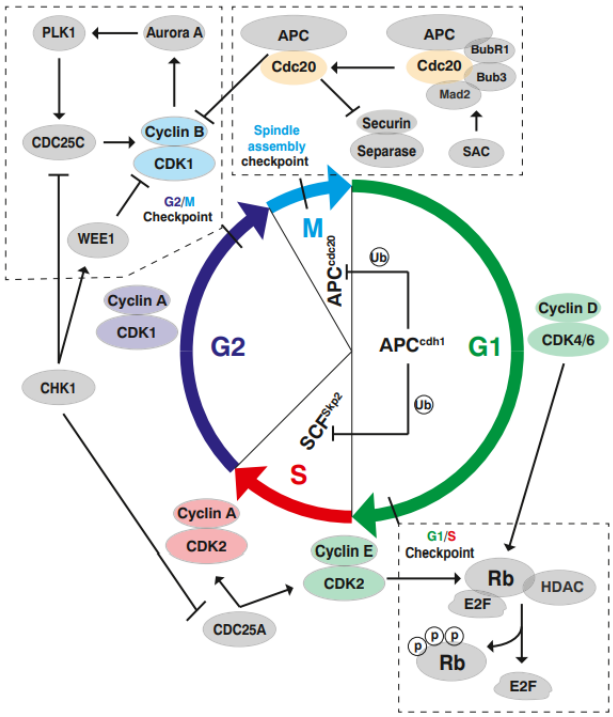

Figure 1. Representative schematic of the cell cycle and checkpoints (Liu *et al*., 2022).

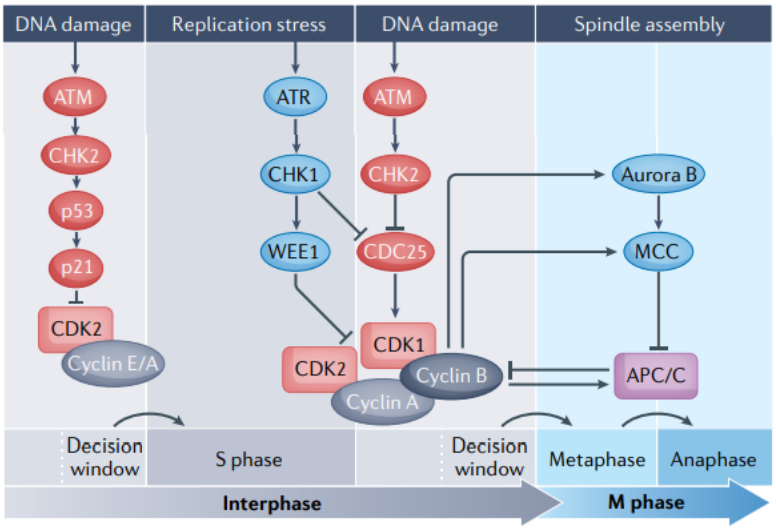

Figure 2. Checkpoint control and the cell cycle (Matthew *et al*., 2022).

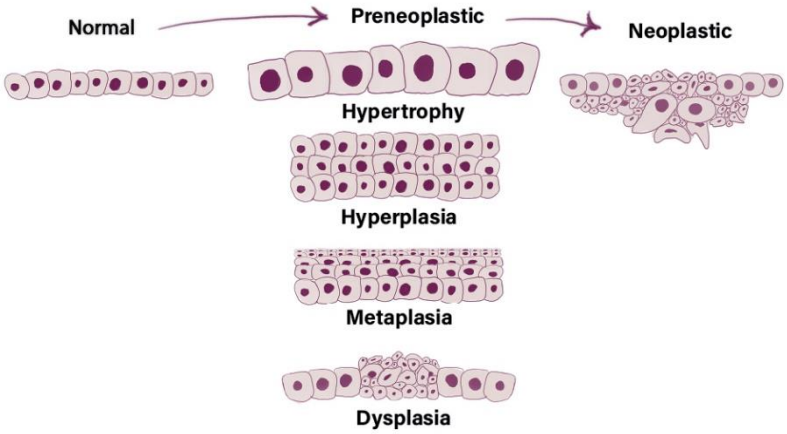

Figure 3. Neoplasia development process (Reference: Author illustration).

Figure 4. Macroscopic appearance of mammary tumors in dogs (Reference: Author documentation).

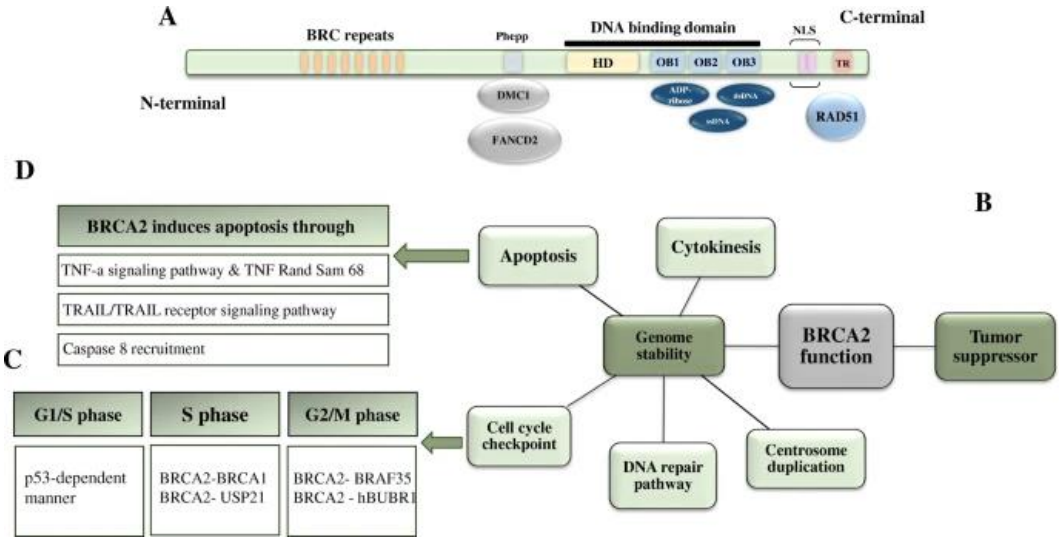

Figure 5. Structure of the BRCA2 gene and the contribution of this protein, especially to the cell cycle and apoptosis pathways (Sadeghi *et al*., 2020).

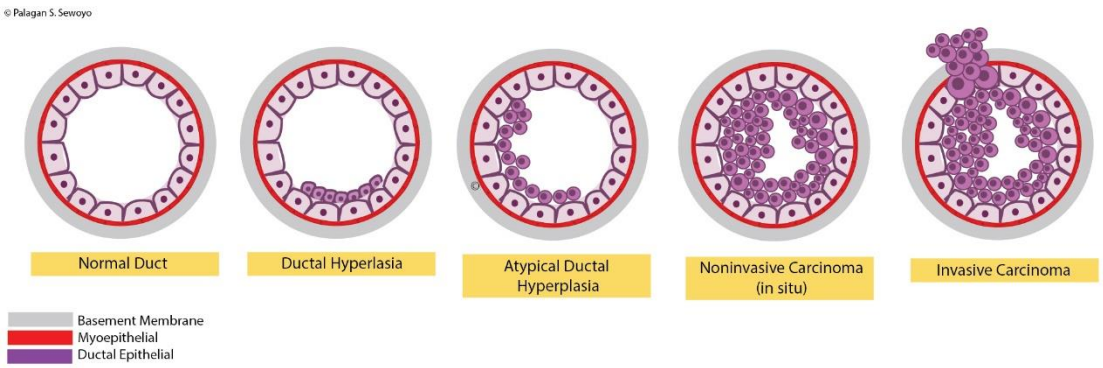

Figure 6. Histological illustration of normal mammary glands, in conditions of hyperplasia, carcinoma in situ and invasive (Reference: Author illustration).

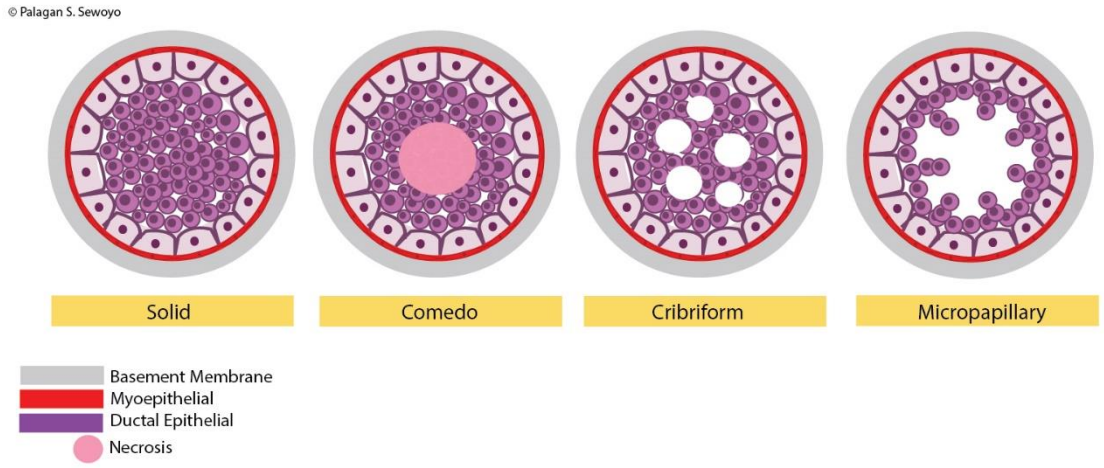

Figure 7. Illustration of the histology and morphology of several types of non-infiltrative carcinoma (Reference: Author illustration).

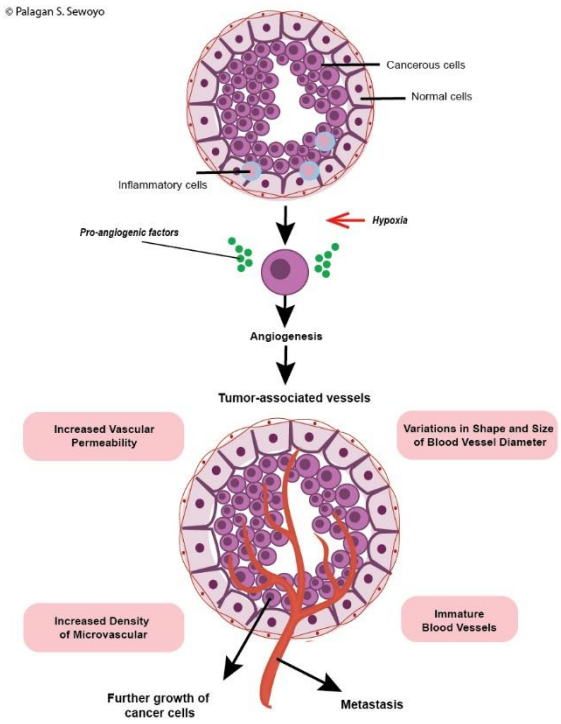

Figure 8. Stages of the angiogenesis process and characteristics of blood vessels in malignant tumor cells (Reference: Author illustration).

Table 1. Histological classification of canine mammary gland tumors (Goldschmidt *et al*., 2011)

Types of Neoplasms	Histological Classification	
Malignant Epithelium	Noninfiltrative carcinoma (in situ)	
	Simple carcinoma	1. Tubular
		2. Tubulopapillary
		3. Cystic-papillary
		4. Cribriform
	Invasive micropapillary carcinoma	
	Solid carcinoma	
	Comedocarcinoma	
	Anaplastic carcinoma	
	Complex carcinomas and adenomas (mixed tumors)	
	Complex type carcinoma (with benign myoepithelial areas)	

	Malignant carcinoma and myoepithelioma Mixed type carcinoma (with areas of myoepithelium and benign mesenchymal tissue) Duct carcinoma Intraductal papillary carcinoma
Malignant Epithelium (Special Type)	Squamous cell carcinoma Adenosquamous cell carcinoma Mucinous carcinoma Lipid-rich carcinoma Spindle cell carcinoma Malignant myoepithelioma Squamous cell carcinoma (spindle cell variant) Spindle cell variant carcinoma Inflammatory carcinoma
Malignant Mesenchymal (Sarcoma)	Osteosarcoma Chondrosarcoma Fibrosarcoma Hemangiosarcoma
Benign	Simple adenomas Intraductal papillary adenoma Basaloid adenoma Fibroadenoma Myoepithelioma Adenomyoepithelioma Benign mixed tumor
Hyperplasia/Dysplasia	Duct ectasia Adenosis Epitheliosis Papillomatosis Fibroadenomatous changes Gynecomastia
Nipple Neoplasm	Adenoma Carcinoma Carcinoma with epidermal infiltration
Nipple Hyperplasia/Dysplasia	Nipple skin melanosis

Table 2. Endogenous regulators of angiogenesis

Activator	Function	Inhibitors	Function
VEGF	Induces angiogenesis, increases vascular permeability	Angiopoietin-2	Antagonistic to Ang1
EGF	Promotes the growth of vascular endothelial cells	Thrombospondin-1,2	Inhibits endothelial growth migration, adhesion, and survival
FGF	Induction of angiogenesis	Collagen	Substrate for MMP
PDGF	Involved in migration of endothelial cells	Endostatin	Inhibits endothelial migration
Angiopoietin-1	Stabilization of vascular endothelium	Angiostatin	Suppresses tumor angiogenesis
TGF	Production of extracellular matrix	TIMP	Suppresses pathological angiogenesis
Ephrin	Controls the formation of blood vessels and lymphatic ducts	Platelet Factor-4	Inhibits the binding of bFGF and VEGF

MMP	Degradation of extracellular matrix, activation of angiogenesis-inducing factors	Vasostatin	Inhibits endothelial growth

VEGF= Vascular Endothelial Growth Factor, EGF= Epidermal Growth Factor, FGF= Fibroblast Growth Factor, PDGF= Platelet-derived Growth Factor, TGF= Transforming Growth Factor, MMP= Matrix metalloproteinase, TIMP= Tissue Inhibitors of Metalloproteinase. Reference: Katayama *et al*. (2019).

(Sadeghi *et al*., 2020). The BRCA2 gene encodes a total of 3418 amino acids for different functional domains (Figure 5). The N-terminal part of BRCA2 contains eight BRC repeats (amino acids 1009–2082) with an estimated 1000 amino acids. The function of the N-terminal part is still unclear, but it is known that the BRC repeat in this area is responsible for interactions between proteins, in particular the interaction between BRCA2 and RAD51. The C-terminal part of BRCA2 contains the BRCA2 DNA-binding domain (amino acids 2478–3185) which consists of a helical domain (HD), three oligonucleotides (OB), and a tower domain (T). The helical domain encodes 190 amino acids and the OB domain consists of OB1, OB2, and OB3, containing approximately 110 amino acids. The OB domain is responsible for BRCA2's affinity for ssDNA and dsDNA damage. The study by Rivera *et al*. (2009) showed that BRCA2 in dogs is closely related to the incidence of benign and malignant mammary tumors. BRCA2 (and also BRCA1) is known to contribute to the risk of mammary tumors in English springer spaniel dogs (Rivera *et al*., 2009). Yoshikawa *et al*. (2015) then investigated BRCA2 levels in mammary tumor samples compared with normal mammary gland samples, which showed that BRCA2 expression was lower in tumor samples. This indicates that low BRCA2 levels contribute to the development of mammary tumors in dogs. The decrease in BRCA2 levels is caused by mutations occurring in the promoter region of the BRCA2 gene, which has previously been reported in human samples (Liu *et al*., 2014). In humans, polymorphisms in one nucleotide of the BRCA2 gene disrupt BRCA2 mRNA expression levels and increase the risk of breast cancer.

In general, genetic variations of BRCA2 in dogs consist of single nucleotide polymorphism (SNP), insertions, and deletions. Exon 11, in both humans and dogs, is the largest exon and encodes the BRC repeat domain, which is a crucial motif for interaction with RAD51. The BRCA2 protein plays a key role in genome stability by recombining DNA and repairing double-stranded DNA breaks. BRCA2 interacts with the RAD51 protein. In dogs, BRCA2 interacts with RAD51 via the BRC repeat (BRC1-8 located in the 11th exon) and the C-terminus. Polymorphisms that occur in BRCA2 result in a decrease in the binding ability of BRC3-4 with RAD51. Further investigation revealed that the binding strength between BRCA2 and RAD51 was strongly influenced by BRC3. This reduction in binding ability results in disruption of the DNA repair process through the homologous recombination pathway (Ozmen *et al*., 2017).

Diagnosis

History, clinical symptoms, and physical examination are the basis for diagnosing diseases, including mammary gland tumors. Routine veterinary procedures such as palpation and general examination of the dog are often performed, and in some cases, a superficial mass will be clearly observed in the mammary gland area (Sorenmo *et al.*, 2020). To confirm the type of tumor and level of malignancy, fine needle aspiration biopsy is generally used for cytological examination. This technique is fast, simple, and inexpensive, but has lower sensitivity and specificity than histopathology. The sensitivity of cytology examination in diagnosing mammary gland tumors is 65–88%, while histopathology is 94–96% (Kaszak *et al*., 2022). For further information, histopathology and

immunohistochemistry techniques are used to classify in more detail the type of tumor and the level of malignancy, thereby producing a definitive diagnosis (Kaszak *et al*., 2022). Prognosis can be determined by macroscopic tumor size, histological type, proliferation index, and clinical stage (Kaszak *et al*., 2022; Kwon *et al*., 2023). Macroscopic tumors that are not less than 3 cm have a better prognosis (Torres *et al*., 2021).

Determination of the Degree of Malignancy (Staging)

Dogs with mammary tumors can be assessed for the stage of malignancy using the TNM system (T: tumor size; N: affected lymph nodes; M: metastasis) modified from WHO. Recently, determining the degree of malignancy was inspired by human oncology, i.e., T was replaced by tumor size (pT), and N was replaced by the pathological status of the lymph nodes (pN), with the addition of lymphovascular invasion (LVI), with several stages, e.g., stage 0, I, II, III A, and III B (Chocteau *et al*., 2019).

Histological Classification

The histological classification of canine mammary gland tumors is based on the classification proposed by Goldschmidt *et al*. (2011). Noninvasive carcinoma (in situ) consists of cancer cells that are in the duct and do not spread to the surrounding mammary tissue. This is different from invasive carcinoma which spreads to surrounding tissue through the basement membrane (Figure 6). Cell shapes vary from cuboidal, round, to polygonal. Meanwhile, simple carcinoma only consists of one type of cell, either epithelial or myoepithelial (Goldschmidt *et al*., 2011).

Tubular, tubulopapillary, cystic papillary, cribriform, and comedo forms, refer to the aggregate form of neoplastic cells (Table 1). In tubular carcinoma, the cells are predominantly arranged in a tubular pattern. This type is most common in dogs. The layer of cells in the tubule is usually 1–2 cells, with varying cell morphology. The tubulopapillary form of the cells is predominantly arranged in a sessile or pedunculated papillary. The difference with tubular carcinoma, this type of carcinoma has papillae that extend into the tubular lumen. These papillae are supported by fibrovascular stroma (Purnama *et al*., 2019). The cystic-papillary type differs slightly from the other tubular types, as its papillae extend into the dilated lumina of the tubular cystic. Cribriform carcinoma is rare, this tumor is characterized by the proliferation of neoplastic cells and appears as small lumens which are usually round (Figure 7).

Micropapillary invasive carcinoma is a type of mammary neoplasm recently described in dogs. This type of neoplasm is characterized by an intraductal population of neoplastic cells that form irregular intraluminal aggregates and small papillae that lack fibrovascular tissue. The cells are pleomorphic and cuboidal to polygonal in shape. This type of tumor shows vascular invasion and has high metastatic potential (Goldschmidt *et al*., 2011). Solid carcinoma is dominated by cells arranged in a dense pattern without any lumina. Comedocarcinoma is characterized by the presence of an area of necrosis in the midst of an aggregate of neoplastic cells. In the area of necrosis, there are eosinophilic amorphous materials mixed with cellular debris, necrotic neutrophils, and macrophages (Goldschmidt *et al*., 2011).

Conventional Therapy of Malignant Mammary Tumors

Currently, surgical resection is the main choice for treating mammary gland carcinoma in dogs followed by adjuvant therapy (Sorenmo *et al*., 2020). In benign cases, surgical resection is sufficient. Lumpectomy and mastectomy are two types of surgical options for removing mammary gland tumors, but this choice is often influenced by certain criteria such as the size of the tumor mass, the number of lesions, and the condition of the surrounding tissue. A lumpectomy is chosen when the nodule measures less than 0.5 cm, and is superficial (Papazoglou *et al*., 2014). Mastectomy is divided into unilateral or bilateral, which is often performed when there are a large number of nodules. However, there is no difference in terms of prognosis between the two

approaches. The best approach is to remove the mass completely with wide surgical margins (Tran *et al*., 2016), followed by adjuvant therapy such as chemotherapy or radiotherapy (Sorenmo *et al*., 2020). In several reports, this procedure has a good prognosis with a smaller chance of recurrence compared to surgery alone. In cases of malignant tumors with lymphatic or vascular invasion, there is a high possibility of recurrence and metastasis, so surgery alone is not able to achieve maximum healing (Tran *et al*., 2016; Sorenmo *et al*., 2020).

It was reported that adjuvant therapy for mammary carcinoma cases using carboplatin and firocoxib after surgery increased survival by 570 days compared to surgery alone, namely 63 days (Lavalle *et al*., 2012). Overall, surgical resection of the tumor is the best approach to the treatment and prevention of canine mammary carcinoma. This procedure involves removing large-diameter tumors along with lymph nodes to prevent recurrence (Sorenmo *et al*., 2020).

Adjuvant therapy using hormones is commonly known to treat cases of human breast cancer. The choice of therapy is based on histopathological assessment with evidence of the presence of hormone receptors. Tamoxifen is an example of an estrogen receptor antagonist drug. This drug interferes with the estrogen signaling pathway by binding to its receptor on malignant tumor cells. Tamoxifen can also be used as a chemopreventive drug in patients with high-risk factors, especially hereditary predisposition. In theory, tamoxifen should work well in canine mammary tumors with positive cases for estrogen receptors (Ahmad, 2018; Ali *et al*., 2016). Evaluation of tamoxifen in canine mammary tumors was carried out by Dileepkumar *et al*. (2015), who described that this drug was able to trigger apoptosis in dog mammary tumors and was declared effective.

Some non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit the production of cyclooxygenase (COX) can prevent the production of prostaglandins. Two forms of COX enzymes, i.e., COX-1 and COX-2, act as analgesics and antipyretics. The study of Millanta *et al*. (2016) revealed that the COX-2 enzyme is expressed in cases of malignant canine mammary tumors. High expression of COX-2 is known to be closely related to a poor prognosis because this enzyme can stimulate tumor angiogenesis, thereby increasing the number of blood vessels and the level of tumor proliferation. It was reported that many cases of canine mammary tumors were positive for expressing COX-2 (Dileepkumar *et al*., 2015; Millanta *et al*., 2016). Repeated use of the newest class of NSAIDs, namely celecoxib, has been reported to be associated with a reduced risk of the emergence and progression of mammary tumors in humans (Li *et al*., 2018). Preclinical studies show that celecoxib is able to suppress cancer proliferation and growth through various mechanisms. Although promising, the study by Chen *et al*. (2014) showed that the use of celecoxib can increase the risk of cardiovascular disease.

Tumor Vascularization

The process of cancer growth requires a very large supply of nutrients in order to reach a larger size. The nutritional supply is channeled through the blood vessels. The formation of new blood vessels is needed to support the rapid proliferation of cancer cells. Since 1971, it has been known that without the growth of new blood vessels, cancer will not grow more than 1–2 mm^3 (Dileepkumar *et al*., 2015; Katayama *et al*., 2019). Initially, neoplastic cells are well-oxygenated through a simple diffusion process called the 'avascular state'. Over time, the tumor will experience a lack of oxygen and undergo phenotypic changes to a pro-angiogenic state (angiogenic switch) (Petrovic, 2016).

One of the proteins that plays a role in the formation of new blood vessels is vascular endothelial growth factor (VEGF). This protein is produced when the tumor experiences hypoxic conditions. VEGF expression is triggered by hypoxia-inducible factor 1 (HIF-1). The presence of new blood vessel growth will provide an opportunity to form a subpopulation of tumor cells with high metastatic potential (Figure 8). This subpopulation of cells is equipped with proteolytic enzymes required for metastasis. This enzyme plays an important role in damaging the

basement membrane and extracellular matrix (Newkirk *et al*., 2017).

Neovascularization is regulated by a balance between factors that trigger angiogenesis (angiogenesis-inducing factors) and factors that inhibit angiogenesis (angiogenesis-inhibiting factors) which are presented in Table 2. Vascular endothelial growth factor (VEGF) plays an important role in the initiation of angiogenesis. The VEGF family consists of five members, including VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor (P1GF). VEGF signals are transmitted via three VEGF receptor tyrosine kinases: VEGFR1, VEGFR2, and VEGFR3 (Katayama *et al*., 2019). The VEGF protein family is a very important factor in triggering neovascularization (Valdivia *et al*., 2021). VEGF triggers the proliferation of endothelial cells, promotes cell migration, and reduces the rate of apoptosis (Triana *et al*., 2020). This protein also increases vascular permeability and promotes migration and circulation of other cells (Apte *et al*., 2019). In the case of mammary cancer, its growth through angiogenesis is directly correlated with VEGF expression (Valdivia *et al*., 2021). VEGF expression in cases of mammary carcinoma in dogs is closely related to the aggressive characteristics of the tumor and poor prognosis (Moscheta *et al*., 2015).

The formation of blood vessels in tumor tissue is not like the formation of blood vessels in wound healing and has unusual morphological characteristics. In normal blood vessels, the distribution of arteries, capillaries, and veins is stable and the vessels are hierarchically sequenced. In comparison, the tumor's angiogenic vessels were dilated and highly tortuous. Vascular thickness and blood vessel diameter are also not uniform (Forster *et al*., 2017). The shape tends to be irregular and branched, without a clear structure. Tumor blood vessels generally grow from existing blood vessels or originate from endothelial cell precursors (Apte *et al*., 2019). The shape of the lumen of tumor blood vessels is irregular, often found without a tunica media layer and only composed of thin walls of endothelial cells (Forster *et al*., 2017; Katayama *et al*., 2019).

Antiangiogenic Therapy

The intravascular characteristics of the tumor are extremely abnormal, immature, and have high permeability. For the treatment of solid tumors, inhibition of angiogenesis is known as a potential target because it can cause hypoxia which results in tumor tissue necrosis, so that its volume decreases (Ramadan *et al*., 2020). Mammary tumors are one of the solid tumors that overexpress VEGF. In both humans (Mohan *et al*., 2021) and dogs (Dos Anjos *et al*., 2019), the expression of VEGFR2 and DGFR is increased in cases of malignant tumors and is closely correlated with the incidence of metastases and the degree of tumor malignancy. In human medicine, approximately one-third of molecular therapies in clinical development are directed against angiogenesis. A widely studied antiangiogenic agent is bevacizumab (monoclonal antibody to anti-VEGFA ligand). In 2008, this drug became the first drug approved by the Food and Drug Administration (FDA) for human breast cancer, but in the end, its authorization was revoked because it was clinically less effective (Li *et al*., 2015). Other drugs, such as pazopanib, are effective in treating kidney carcinoma, soft tissue sarcoma, and breast cancer in combination with anti-HER-2 lapatinib (Slamon *et al*., 2008), while in veterinary medicine, sorafenib has been tested and has quite promising potential. In vitro, sofarenib was able to inhibit vasculogenic mimicry (VM) in canine mammary tumor cells (Prado *et al*., 2019). The drugs rivoceranib (apatinib) (Slamon *et al*., 2008) and vandetanib (Kennedy *et al*., 2011) targeting VEGFR2 also showed quite promising results based on in vitro tests on canine mammary tumor cell lines.

Apart from drugs, there are viruses that can be used for cancer therapy that inhibit angiogenesis and target genes that regulate angiogenesis. A report by Al-Shammari *et al*. (2022) showed that the Newcastle Disease Virus (NDV) AMHA1 strain from Iraq was able to suppress angiogenesis in a mammary adenocarcinoma model. The histopathological figure shows that the number of tumor blood

vessels has decreased. Furthermore, this virus is able to change the expression of proteins involved in angiogenesis, namely angiopoietin-1, angiopoietin-2, and EGF. These protein levels tend to decrease compared to animals that are not treated with this virus. Angiopoietin plays a very important role in coordinating with VEGF to form new vascular growth. Apart from the strain originating from Iraq, the NDV strain from Indonesia, namely Tabanan-1/ARP/2017 (GenBank Accession Number: MH215997.1), also has potential as a cancer therapy that inhibits angiogenesis. The study of Sewoyo *et al*. (2021) showed that the NDV Tabanan-1/ARP/2017 isolate was able to reduce the number of blood vessels in rat fibrosarcoma models. Through immunohistochemical examination of Ki67, VEGF, TNF-α, and TGF-β, the NDV Tabanan-1/ARP/2017 isolate was able to reduce the expression of these proteins (Adi *et al*., 2023). Naturally, this is encouraging news, and given the high frequency of mammary cancers in dogs and the limited number of available therapy options, it is hoped that more study on mammary tumor models will be conducted.

CONCLUSION

Dog mammary tumors and human breast tumors are comparable. Mammary tumorigenesis is a closely related process to a number of causes. For dogs with malignant mammary tumors, combined therapy is required, much like in people. Due to the immune system's cell defense systems and the tumor's immunosuppression, there is currently no certainty that malignant mammary tumors in dogs can be entirely treated. Targeting and inhibiting the genes involved in tumor angiogenesis is one of the treatment approaches that require more study to be developed.

ACKNOWLEDGEMENTS

The author expresses gratitude to Udayana University for providing resources for literature searches.

REFERENCES

Adi, A. A. A. M., Putra, I. G. A. A., & Sewoyo, P. S. (2023). Potensi Virus Newcatle Disease untuk Viroterapi Kanker. Penerbit Deepublish.

Ahmad, I. (2018). Tamoxifen a pioneering drug: An update on the therapeutic potential of tamoxifen derivatives. *European journal of medicinal chemistry*, 143, 515–531.

Ali, S., Rasool, M., Chaoudhry, H., Pushparaj, P. N., Jha, P., Hafiz, A., & Jamal, M. S. (2016). Molecular mechanisms and mode of tamoxifen resistance in breast cancer. *Bioinformation*, 12(3), 135.

Allard, P. (2014). Bisphenol A. Biomarkers in Toxicology, pp: 259–474.

Al-Shammari, A. M., Al-Mudhafr, M. A., Al-Grawi, E. C., Al-Hili, Z. A., & Yaseen, N. (2022). Newcastle disease virus suppresses angiogenesis in mammary adenocarcinoma models. *Bulgarian Journal of Veterinary Medicine*, 25(1).

Apte, R. S., Chen, D. S., & Ferrara, N. (2019). VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell*, 176, 1248–1264.

Astawa, I. N. M. (2018). Dasar-dasar Patobiologi Molekuler I: Apoptosis & Onkogenesis. Airlangga University Press.

Brunelle, M., Sartin, E. A., Wolfe, L. G., Sirois, J., & Dore, M. (2006). Cyclooxygenase-2 expression in normal and neoplastic canine mammary cell lines. *Veterinary Pathology*, 43(5), 656–666.

Canadas-Sousa, A., Santos, M., Leal, B., Medeiros, R., & Dias-Pereira, P. (2019). Estrogen receptors genotypes and canine mammary neoplasia. *BMC Veterinary Research*, 15, 1–10.

Canaud, G., & Bonventre, J. V. (2015). Cell cycle arrest and the evolution of chronic kidney disease from acute kidney injury. *Nephrology Dialysis Transplantation*, 30(4), 575–583.

Chen, J., Shen, P., Zhang, X. C., Zhao, M. D., Zhang, X. G., & Yang, L. (2014). Efficacy and safety profile of celecoxib for treating advanced cancers: a meta-analysis of 11 randomized clinical trials. *Clinical Therapeutics*, 36, 1253–1263.

Chocteau, F., Abadie, J., Loussouarn, D., & Nguyen, F. (2019). Proposal for a histological staging system of mammary carcinomas in dogs and cats. Part 1: canine mammary carcinomas. *Frontiers in Veterinary Science*, 6, 388.

Dileepkumar, K. M., Maiti, S.K., Kumar, N., & Shams-uz-Zama, M. M. (2015). Therapeutic Evaluation of Anti-angiogenic and Chemotherapy with or without Cox-2 Inhibitor and Immunomodulator Drug in the Management of Canine Mammary Neoplasm. *Pakistan Veterinary Journal*, 35, 365–370.

Dos Anjos, D. S., Vital, A. F., Lainetti, P. D. F., Leis-Filho, A. F., Dalmolin, F., Elias, F., & Fonseca-Alves, C. E. (2019). Deregulation of VEGFR-2 and PDGFR expression and microvascular density in a triple-negative model of canine malignant mammary tumors with lymph node or lung metastasis. *Veterinary Sciences*, 6(1), 3.

Ellinger, I., & Ellinger, A. (2014). Smallest unit of life: cell biology. *Comparative Medicine: Anatomy and Physiology*, 19–33.

Enginler, S. O., Akış, I., Toydemir, T. S. F., Oztabak, K., Haktanir, D., Gündüz, M. C., & Fırat, I. (2014). Genetic variations of BRCA1 and BRCA2 genes in dogs with mammary tumours. *Veterinary Research Communications*, 38, 21–27.

Forster, J. C., Harriss-Phillips, W. M., Douglass, M. J. J., & Bezak, E. (2017). A review of the development of tumor vasculature and its effects on the tumor microenvironment. *Hypoxia*, 5, 21–32.

Goldschmidt, M., Peña, L., Rasotto, R., & Zappulli, V. (2011). Classification and grading of canine mammary tumors. *Veterinary Pathology*, 48(1), 117–131.

Gray, M., Meehan, J., Martinez-Perez, C., Kay, C., Turnbull, A. K., Morrison, L. R., Pang, L. Y., & Argyle, D. (2020). Naturally-Occuring Canine Mammary Tumors as a Translational Model for Human Breast Cancer. *Frontiers in Oncology*, 10, 617.

Hanahan, D. (2022). Hallmarks of cancer: new dimensions. *Cancer Discovery*, 12(1), 31–46.

Kaszak, I., Witkowska-Pilaszewicz, O., Domrazek, K., & Jurka, P. (2022). The Novel Diagnostic Techniques and Biomarkers of Canine Mammary Tumors. *Veterinary Sciences*, 9, 526.

Katayama, Y., Uchino, J., Chihara, Y., Tamiya, N., Kaneko, Y., Yamada, T., & Takayama, K. (2019). Tumor neovascularization and developments in therapeutics. *Cancers*, 11(3), 316.

Kennedy, K. C., Qurollo, B. A., Rose, B. J., & Thamm, D. H. (2011). Epidermal growth factor enhances the malignant phenotype in canine mammary carcinoma cell lines. *Veterinary and Comparative Oncology*, 9(3), 196–206.

Komazawa, S., Sakai, H., Itoh, Y., Kawabe, M., Murakami, M., & Mori, T. (2016). Canine tumor development and crude incidence of tumors by breed based on domestic dogs in

Gifu prefecture. *Journal Veterinary Medical Science*, 78, 1269–1275.

Kristiansen, V. M., Peña, L., Díez Córdova, L., Illera, J. C., Skjerve, E., Breen, A. M., & Sørenmo, K. U. (2016). Effect of ovariohysterectomy at the time of tumor removal in dogs with mammary carcinomas: a randomized controlled trial. *Journal of Veterinary Internal Medicine*, 30(1), 230–241.

Kwon, J. Y., Moskwa, N., Kang, W., Fan, T. M., & Lee, C. (2023). Canine as Comparative and Translational Model for Human Mammary Tumor. *Journal of Breast Cancer*, 26, 1–13.

Lavalle, G. E., De Campos, C. B., Bertagnolli, A. C., & Cassali, G. D. (2012). Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. *In vivo*, 26(3), 375–379.

Li, J., Hao, Q., Cao, W., Vadgama, J. V., & Wu, Y. (2018). Celecoxib in breast cancer prevention and therapy. *Cancer Management and Research*, 10, 4653–4667.

Li, Q., Yan, H., Zhao, P., Yang, Y., & Cao, B. (2015). Efficacy and safety of bevacizumab combined with chemotherapy for managing metastatic breast cancer: a meta-analysis of randomized controlled trials. *Scientific Reports*, 5(1), 1–11.

Liu, J., Peng, Y., & Wei, W. (2022). Cell cycle on the crossroad of tumorigenesis and cancer therapy. *Trends in Cell Biology*, 32(1), 30–44.

Matthews, H. K., Bertoli, C., & de Bruin, R. A. (2022). Cell cycle control in cancer. *Nature Reviews Molecular Cell Biology*, 23(1), 74–88.

Meuten, D. J. (2016). Tumor in Domestic Animals. Hoboken, NY: John Wiley and Sons.

Michishita, M. (2020). Understanding of tumourigenesis in canine mammary tumours based on cancer stem cell research. *The Veterinary Journal*, 265, 105560.

Millanta, F., Asproni, P., Canale, A., & Poli, A. (2016). COX-2, mPGES-1, and EP2 receptor immunohistochemical expression in canine and feline malignant mammary tumors. *Veterinary Comparative Oncology*, 14, 270–280.

Misdorp, W. (2002). Tumors of the mammary gland. Tumors in domestic animals, pp: 575–606.

Mohan, N., Luo, X., Shen, Y., Olson, Z., Agrawal, A., Endo, Y., Rotstein, D. S., Pelosof, L. C., & Wu, W. J. (2021). A Novel Bispecific Antibody Targeting EGFR and VEGFR2 Is Effective against Triple Negative Breast Cancer via Multiple Mechanisms of Action. *Cancers*, 13, 1027.

Moschetta, M. G., Maschio, L. B., Jardim-Perassi, B. V., Gelaleti, G. B., Lopes, J. R., Leonel, C., & Zuccari, D. A. P. D. C. (2015). Prognostic value of vascular endothelial growth factor and hypoxia-inducible factor 1α in canine malignant mammary tumors. *Oncology Reports*, 33(5), 2345–2353.

Newkirk, K. M., Brannick, E. M., & Kusewitt, D. F. (2017). Neoplasia and tumor biology. In Pathologic basis of veterinary disease. Mosby. pp: 286–321.

Ozmen, O., Kul, S., Risvanli, A., Ozalp, G., Sabuncu, A., & Kul, O. (2017). Somatic SNPs of the BRCA2 gene at the fragments encoding RAD51 binding sites of canine mammary tumors. *Veterinary and Comparative Oncology*, 15(4), 1479–1486.

Papazoglou, L. G., Basdani, E., Rabidi, S., Patsikas, M. N., & Karayiannopoulou, M. (2014). Current surgical options for mammary tumor removal in dogs. *Journal Veterinary Science Medicine*, 2(1), 6.

Petrovic, N. (2016). Targeting angiogenesis in cancer treatments: where do we stand?. *Journal of Pharmacy & Pharmaceutical Sciences*, 19(2), 226–238.

Prado, M. C. M., Macedo, S. D. A. L., Guiraldelli, G. G., de Faria Lainetti, P., Leis-Filho, A. F., Kobayashi, P. E. & Fonseca-Alves, C. E. (2019). Investigation of the prognostic significance of vasculogenic mimicry and its inhibition by sorafenib in canine mammary gland tumors. *Frontiers in Oncology*, 9, 1445.

Purnama, M. T. E., Rahmaningtyas, I. H., Pratama, A. R., Prastika, Z., Kartikasari, A. M., & Cahyo, N. P. D. (2019). Tadpole serum activity (*Rana catesbeiana*) in caspase-3 as a marker of the role of apoptosis and total cytotoxic T lymphocytes in albino rats' epithelial cells induced by neoplasia. *Veterinary World*, 12(1), 63.

Ramadan, W. S., Zaher, D. M., Altaie, A. M., Talaat, I. M., & Elmoselhi, A. (2020). Potential therapeutic strategies for lung and breast cancers through understanding the anti-angiogenesis resistance mechanisms. *International Journal of Molecular Sciences*, 21(2), 565.

Rivera, P., Melin, M., Biagi, T., Fall, T., Häggström, J., Lindblad-Toh, K., & von Euler, H. (2009). Mammary tumor development in dogs is associated with BRCA1 and BRCA2. *Cancer Research*, 69(22), 8770–8774.

Sadeghi, F., Asgari, M., Matloubi, M., Ranjbar, M., Karkhaneh Yousefi, N., Azari, T., & Zaki-Dizaji, M. (2020). Molecular contribution of BRCA1 and BRCA2 to genome instability in breast cancer patients: Review of radiosensitivity assays. *Biological Procedures Online*, 22, 1–28.

Sewoyo, P. S., Adi, A. A. A. M., Winaya, I. B. O., Sampurna, I. P., & Bramardipa, A. A. B. (2021). The Potency of Virulent Newcastle Disease Virus Tabanan-1/ARP/2017 As Virotherapy Agents in Rat Fibrosarcoma Models. *Journal of Advanced Veterinary Research*, 11(2), 64–68.

Slamon, D., Gomez, H. L., Kabbinavar, F. F., Amit, O., Richie, M., Pandite, L., & Goodman, V. (2008). Randomized study of pazopanib+ lapatinib vs. lapatinib alone in patients with HER2-positive advanced or metastatic breast cancer. *Journal of Clinical Oncology*, 26(15), 1016–1016.

Sorenmo, K. U., Rasotto, R., & Zappulli, V. (2020). Tumors of the mammary gland. In Vaild, D., Thamm, D., Liptack, J. (Ed). Withrow and MacEwen's Small Animal Clinical Oncology. 6 ed. St Louis, MO: Saunders, pp: 604–625.

Thumser-Henner, P., Nytko, K. J., & Rohrer Bley, C. (2020). Mutations of BRCA2 in canine mammary tumors and their targeting potential in clinical therapy. *BMC Veterinary Research*, 16(1), 1–9.

Torres, C. G., Iturriaga, M. P., & Cruz, P. (2021). Hormonal carcinogenesis in canine mammary cancer: Molecular mechanisms of estradiol involved in malignant progression. *Animals*, 11(3), 608.

Tran, C. M., Moore, A. S., & Frimberger, A. E. (2016). Surgical treatment of mammary carcinomas in dogs with or without postoperative chemotherapy. *Veterinary Comparative Oncology*, 14, 252–262.

Triana, N. M., Wilujeng, E., Putri, M. W. H., Yuda, D. M. P., Hardiono, A. L., Purnama, M. T. E., & Fikri, F. (2020).

Antiproliferation effects of *Glycine max Linn* ethanolic extract on induced mammary gland carcinoma in albino rats. *In IOP Conference Series: Earth and Environmental Science*, 441(1), 012103.

Valdivia G., Alonso-Diez, A., Perez-Alenza, D., & Pena, L. (2021). From Conventional to Precision Therapy in Canine Mammary Cancer: A Comprehensive Review. *Frontiers in Veterinary Science*, 8, 623800.

Vascellari, M., Capello, K., Carminato, A., Zanardello, C., Baioni, E., & Mutinelli, F. (2016). Incidence of mammary tumors in the canine population living in the Veneto region (Northeastern Italy): Risk factors and similarities to human breast cancer. *Preventivce Veterinary Medicine*, 126, 183–189.

Widyarini, S., Sugiyono, Kristianingrum, Y. P., & Sutrisno, B. (2022). Studi Histopatologis Tumor pada Hewan Kesayangan dan Hewan Liar. *Jurnal Sain Veteriner*, 40, 73–84.

Wooster, R., Bignell, G., Lancaster, J., Swift, S., Seal, S., Mangion, J., & Stratton, M. R. (1995). Identification of the breast cancer susceptibility gene BRCA2. *Nature*, 378(6559), 789–792.

Yoshikawa, Y., Morimatsu, M., Ochiai, K., Ishiguro-Oonuma, T., Wada, S., Orino, K., & Watanabe, K. (2015). Reduced canine BRCA2 expression levels in mammary gland tumors. *BMC Veterinary Research*, 11, 1–8.

***