Review

# Molecular Mechanisms of Canine Cancers

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**Abstract**

Cancer is the leading cause of death in dogs, and 50 percent of dogs over the age of 10 develop cancer at some point. The most common cancers in dogs include lymphoma, mast cell tumors, osteosarcoma, mammary gland tumors, and melanoma, and many of them share marked similarities with their human counterparts. Although canines are afflicted with many of the same types of cancers as humans, the genetic basis behind these cancers are not as well understood. Thus, the aim of this study is to elucidate some of the molecular mechanisms behind canine cancers. Canine lymphoma mutation patterns generally vary with the type of lymphoma afflicted–B-cell lymphomas have mutations in the alternative NF-kB pathway including MAP3K14, whereas in T-cell lymphomas the mTOR pathway in boxers and cellular metabolism genes in golden retrievers are affected. Mast cell tumors are largely traced to internal tandem duplications and deletions in the juxtamembrane domain of the proto-oncogene c-KIT. In osteosarcoma, mutations in RB1 and TP53 (especially G: C->A:T

transitions in exons 4 and 5), as well as CDK4 inhibitors CDKN2A/B are common. Mammary gland tumors are associated with BRCA2 underexpression due to reading frame shift and mutations in BRC repeat 3. Lastly, deletion or underexpression of p16 and PTEN and altered expression of cell–cell adhesion molecules are common factors in the development of melanoma. The genes identified were then studied to identify more key amino acid mutations that changed protein products and promoted tumorigenesis. Genes that altered expression levels of proteins were analyzed separately. Both sets of candidate genes were then analyzed with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) in order to elucidate the molecular pathways involved in canine cancers and identify more genes possibly involved in tumorigenesis. The proposition of this review is that treatments for both canine and human cancers would be enhanced by comparative genomic studies.

## 1. Introduction

As the leading cause of death in dogs, cancer affects approximately one in three dogs [1]. The incidence of cancer among dogs is similar to the incidence of cancer in humans: for reference, cancer affects approximately one in two men and one in three women [2]. Indeed, nearly half of all dogs over the age of ten will develop cancer. Although many of the most common canine cancer types—lymphoma, mast cell tumors, osteosarcoma, mammary gland tumors, and melanoma—are similar to human cancers, they remain much less understood. In addition, canines are a promising model for human cancers, as they age faster than humans but share the same environments and have high levels of phenotypic diversity [3]. Raposo and colleagues emphasized that canine inflammatory mammary carcinoma (CIMC) can serve as a very good model of human inflammatory breast cancer (IBC) [4-6].

Lymphoma and mammary gland tumors are two of the most common canine neoplasms. Lymphoma accounts for approximately 7%–21% of all canine cancer cases, while mammary gland tumors represent at least half of all cancers in female dogs. In a study conducted on canine cancer frequency in Genoa, Italy, the incidence of mammary gland tumors was found to be 70% of female canine cancer cases [7]. Because of the high rate of mammary gland tumors among female dogs, total cancer incidence was found to be three times higher in female than male dogs. Lymphoma was found in approximately equal incidence rates in male and female dogs. Skin neoplasms were the second most common types of cancers in male dogs [7]. In particular, melanoma accounts for 9%–20% of skin neoplasms and 4%–7% of all cancers. Mast cell tumors are also a common skin neoplasm, accounting for around 7%–21% of subcutaneous tumors. Osteosarcoma is the most common bone tumor but is much more prevalent in dogs than it is in humans.

Many of these cancers are treated with a combination of chemotherapy, surgery and radiation therapy. However, even with aggressive treatment, prognosis can remain grave. The expected survival time for dogs with B-cell lymphoma is 12 months, while the expected survival time for

dogs with the more aggressive T-cell lymphoma is 6 months [8]. Approximately 20% of dogs will survive more than two years [9-11]. The prognosis for osteosarcoma is similarly poor: Unless early and intense treatment is started upon detection, the average survival time is 3 months without treatment [12-14]. Canines with malignant melanoma have a median survival time ranging from three to 18 months, depending on the stage at diagnosis [15]. Surgery can often stop benign mammary tumors and even malignant tumors, if these are in local clinical stage, but survival time depends heavily on the size and location of the tumor and the age of the dog. Lastly, for mast cell tumors, surgical removal and chemotherapy will effectively treat many tumors, with median survival 1359 days and medial disease-free interval 2120 days [16].

Systemic anticancer chemotherapy is the treatment of choice for canine lymphoma [9, 10, 12, 17]. Many different drugs have been utilized either singly or in combination for the treatment of lymphomas in dogs. The most effective chemotherapy protocol is the CHOP protocol, which utilizes three cytotoxic chemotherapy drugs (cyclophosphamide, doxorubicin and vincristine) in combination with prednisone [9]. This protocol is often considered the "gold standard" for the treatment of lymphoma in dogs. Other treatment options include the COP chemotherapy protocol (cyclophosphamide, doxorubicin, vincristine and prednisone) or single-agent doxorubicin [9]. The most effective protocol reported was sequential combination chemotherapy including lasparaginase, vincristine, cyclophosphamide, and doxorubicin, in conjunction with decreasing doses of prednisone [17]. Future directions for treatment with lymphoma include immunotherapy with vaccines. Bone marrow transplant or treatment is available at certain centers [17]. In remission dogs are treated with a consolidation protocol that consisted of either additional chemotherapy drugs (mechlorethamine, vincristine, procarbazine, prednisone, and lomustine) or half-body radiation therapy [12].

The most effective management of canine appendicular osteosarcoma involves the incorporation of multimodal therapy to address the primary tumor and metastatic disease [14, 18]. Palliative and curative-intent treatments are used for the bilateral synchronous appendicular bone tumors [19]. Beside palliative radiation therapy stereotactic radiation therapy (SRT) and a surgical limb-salvage performed followed by carboplatin chemotherapy is used [18, 19].

Aggressive surgical removal of the mast cell tumor and surrounding tissue is generally the treatment of choice [20, 21] might be highly effective. Radiation therapy might be highly effective as well as multimodality therapy. Several different drugs can be used to treat these high-risk patients, including high doses of steroids, traditional chemotherapy (vinblastine, lomustine) or RTK-inhibitors (Palladia, Kinavet) [21]. In some cases, oral toceranib phosphate (Palladia, SU11654) administration applied [22]. Immunotherapy and vaccination are also used.

The recommended treatment for melanoma consists of local tumor control through surgery and/or radiation therapy, as well as systemic treatment, including immunotherapy and oncolytic virotherapy [23]. Vaccination with oncept is also used [24].

Surgery is suggested as a treatment of choice for all bitches with mammary tumors except those with inflammatory carcinomas [25-27]. Nunes and coauthors point that the surgery can be successful for benign mixed tumors and early-stage (I–III) carcinomas in mixed mammary tumors [25]. As drug therapy is suggested administering doxorubicin combined with cyclophosphamide, doxorubicin combined with carboplatin, carboplatin combined with gemcitabine, and paclitaxel as a single agent [28]. As pointed by Lavalle and colleagues, complementation with adjuvant chemotherapy, results in increased survival compared with bitches undergoing only surgical

excision [29]. Disease-free survival increase in bitches with combination of 5-fluoracil therapy with surgery is reported by Karayannopoulou and colleagues [30].

Below we elucidate the possible molecular mechanisms of five most frequent canine cancers: lymphoma, mast cell tumors, osteosarcoma, mammary gland carcinoma, and melanoma.

## 2. Lymphoma

Canine lymphoma accounts for around 6% of all canine malignancies, making it one of the most common canine cancers [8]. It is more prevalent in older, larger dogs. Boxers, bull mastiffs, basset hounds, golden retrievers, Saint Bernards, Scottish terriers, Airedales and bulldogs are more predisposed to the disease [9]. Small-breed dogs (< 15 kg BM) appear to be affected with lymphoma at a greater age [11]. The most common symptoms include anorexia, weight loss, ascites (abnormal accumulation of abdomen fluid), dyspnea (difficulty in breathing), polydipsia (abnormal thirst), polyuria (excessive urination), and fever [11]. Typical human lymphoma is classified into Hodgkin's lymphoma or non-Hodgkin's lymphoma depending on the presence of the Reed-Sternberg cell, but most canine lymphomas are indistinguishable from non-Hodgkin's lymphoma. Lymphoma subtypes are classified according to their anatomical location, cytomorphology, immunophenotype, genetic, molecular, and clinical features, which results in at least several dozen distinct subtypes of lymphoma [8]. Lymphomas are most broadly separated into B-cell and T-cell lymphomas, of which B-cell is the more common and less aggressive form. B-cell lymphomas make up around 70% of canine lymphoma cases and nearly 90% of human lymphoma cases [8, 31-33]. DLBCL, the most common form of B-cell lymphoma, accounts for over half of canine lymphomas and a fifth of human lymphomas [8]. Peripheral T-cell lymphoma not otherwise specified (PTCL, NOS), is the second most common subtype of canine lymphoma and the most common form of canine T-cell lymphoma, but is significantly less frequent in humans [8]. Even within this subtype, it is difficult to fully characterize the lymphoma, because PTCL, NOS is a general term that can be further divided by the T-cells they originate from: cytotoxic, helper, and follicular helper PTCL [8]. If classified according to anatomical location, multicentric lymphoma, which affects the peripheral lymph nodes, is most common.

Due to the large variety of subtypes of lymphoma, it is difficult to generalize genetic causes and outcomes for canine lymphoma without considering the subtype of lymphoma. In fact, "indolent" lymphomas, including mantle cell lymphoma and marginal zone lymphoma, have a slower rate of progression than other types of lymphomas. As a result, it is also difficult to predict typical survival time for dogs afflicted with lymphoma. Survival time is around 10–12 months for typical B-cell lymphoma and 6 months for T-cell lymphoma, reflecting the trend that T-cell lymphomas tend to be more aggressive. However, dogs with "indolent" lymphomas have approximate survival times of 33 months for T-cell and 21 months for B-cell, reversing this trend [8]. Thus, the review will discuss findings that may be specific to a subtype of canine lymphoma.

According to one study done on 61 dogs on chromosomal aberrations in multicentric lymphoma, 70% of dogs examined showed genomic imbalance as a result of aneuploidy, and the remaining 30% of dogs showed balanced translocations [31]. An extension of this study found that a gain of canine chromosome 13 was the most common aberration, making up 12 out of the 25 cases studied. Gain of chromosome 31 represented eight cases and loss of chromosome 14 represented 5 of the cases [31]. Canine chromosome 13 corresponds to sites 8q23-qtel and

4pprox-qprox on the human chromosome, which carry the MYC oncogene and c-KIT oncogene respectively and may explain its importance in tumorigenesis. However, these genomic imbalances may not be specific to lymphoma as they were also found in other types of tumors, suggesting that aneuploidy may just be a factor in general tumor progression.

Exons 4–8 of the tumor suppressor gene p53 were also examined for possible mutations (Table 1) [extracted from reference 34]. The study found that out of 43 dogs with various anatomical subtypes of lymphoma, 7 dogs (16%) had mutations in those exons. More specifically, one dog had a mutation in exons 4, 5 and 6, and two dogs each had a mutation in exon 7 and 8 [34]. Of the seven dogs with a p53 mutation, three had a single base insertion and four had a single base substitution—one of the dogs had a synonymous substitution, but all the other substitutions resulted in a changed amino acid sequence. The study suggests that p53 mutations may not be indicative of a specific anatomical subtype of lymphoma [34]. Furthermore, the study did not account for different histologic or immunophenotypic subtypes, so further exploration with more dogs and more detailed subtype classification is needed to determine a correlation between p53 mutations and subtype of lymphoma.

**Table 1** Mutations in exons 4–8 of p53 in canine lymphoma.

| Gene | Exon | Nucleotide Mutation | Amino Acid Mutation | Reference |
|------|------|---------------------|---------------------|-----------|
| p53 | 4 | 287_288insT | | 34 |
| p53 | 5 | 434C>T | R145H | 34 |
| p53 | 6 | 603T>A | R201R | 34 |
| p53 | 7 | 679T>C | N227D | 34 |
| p53 | 7 | 687_688insC | | 34 |
| p53 | 8 | 812C>T | R271Q | 34 |
| p53 | 8 | 796_797insA | | 34 |
| TRAF3 | | | R159* | 35 |
| TRAF3 | | | W488* | 35 |
| TRAF3 | | | R423* | 35 |
| TRAF3 | | | W420* | 35 |
| TRAF3 | | | E271* | 35 |
| TRAF3 | | | R360* | 35 |
| TRAF3 | | | H507Q | 35 |
| TRAF3 | | | Q492* | 35 |
| TRAF3 | | | R360* | 35 |
| TRAF3 | | | Y452* | 35 |
| TRAF3 | | | Y449* | 35 |
| TRAF3 | | | E35* | 35 |

Because certain breeds of dog are more susceptible to lymphoma, breed-specific genetic risk factors and mutations were also examined. Three breeds—the boxer, golden retriever and cocker spaniel—predisposed to T-cell, B-cell, and both types of lymphoma respectively, were examined for somatic mutations. The study found that B-cell lymphomas in the golden retriever and cocker spaniel had similar genetic mutations. Known for participation in classical Hodgkin's lymphoma, inhibitory factor TRAF3 and one of the main signaling genes of the alternative NF-kB pathway—MAP3K14, were mutated in 28% of all cases (golden retrievers and cocker spaniels, 64 total), FBXW7 in 25%, and POT1 in 17% of all cases (Table 2) [32, 36]. Most mutations in TRAF3 seemed to affect reading frame or splicing, leading to a loss of TRAF3 protein [35]. Other mutated genes include the tumor suppressor p53 as mentioned above, the gene coding for uncharacterized protein FAM90A1, the RNA helicase DDX3X, proteasome subunit PSMA1, proline-rich nuclear receptor coactivator 1 (PNRC1), and SET-domain containing 2 (SETD2). However, T-cell lymphomas were different in their mutation patterns depending on the breed of dog affected. The T-cell predisposed boxers were typically mutated in their mTOR pathway important in cell-cycle regulation and proliferation, while golden retrievers had mutations in genes relating to cellular metabolism. More specifically, boxers had mutations in SATB1 (25% of 16 cases) and PTEN (25%) [32]. In particular, PTEN seems to be specific to boxer T-cell lymphoma because no golden retrievers had mutations in that gene, and 44% of boxers had mutations in the PI3K–AKT–mTOR pathway, where PTEN acts as an inhibitor (Figure 1) [reproduced from reference 37]. The most significantly mutated genes in golden retriever T-cell lymphomas were PSMA1 (16% of 25 cases), the cytochrome C oxidase subunit involved in apoptosis COX8A (12%), leukotriene A4 hydrolase LTA4H (16%), and the gene coding for TBC1D26 (20%) [32]. SATB1 was the only gene mutated in both kinds of T-cell lymphoma as it was also mutated in 12% of golden retriever T-cell lymphomas. Furthermore, only seven genes were determined to be mutated in both T-cell and B-cell lymphomas, including PSMA1 and the genes for FAM90A1 and TBC1D26 [32].

**Table 2** Gene aberrations and expression changes in canine lymphoma.

| Lymphoma Subtype | Gene | Function | Aberration or/and expression | References |
|---|---|---|---|---|
| multicentric | CFA13 region including NDRG1 gene | NDRG1 is a member of the protooncogene family N-Myc involved in stress and hormone responses | increased expression as a result of gain of chromosome in 48% of 25 cases studied | 31 |
| | CFA31 region including SOD1 gene | SOD1—superoxide dismutase, which breaks down superoxide radicals | increased expression as a result of gain of chromosome in 32% of 25 cases studied | 31 |
| | CFA14 region including SPAM1 gene | SPAM1—sperm adhesion molecule, allows for sperm to penetrate layer covering oocyte | decreased expression as a result of loss of chromosome in 20% of 25 cases studied | 31 |
| | TP53 | tumor suppressor | single base substitutions, inversions and synonymous | 34 |

| | | | substitutions in exons 4–8: 16% of 43 had p53 mutations, 84% did not | |
|---|---|---|---|---|
| DLBCL | DAPK1 | tumor suppressor | hypermethylated | 38 |
| | NFKB1 | DNA transcription and cell survival, mutation is feature of many tumors | increased expression | 39 |
| | IGH | immunoglobulin heavy chain | commonly deleted in B-cell lymphoma—deletions are not usually cancer driven but reflect tumor cell of origin | 32, 39 |
| | TRAF3 | negative regulator of NF-kB | mutations affect reading frame or splicing, leading to loss of TRAF3, mutations present in 20.3% of samples studied | 32, 35 |
| | FBXW7 | targets cyclin E for degradation and controls stability of MYC, a proto-oncogene | mutations present in 25% of samples | 32 |
| | POT1 | protein important for telomere maintenance | mutations present in 17% of samples | 32 |
| | FAM90A1 | uncharacterized protein | mutations present in 15.6% of samples | 32 |
| | TP53 | tumor suppressor | mutations present in exons 5-8, present in 15.6% of samples | 32 |
| | DDX3X | RNA helicase | mutations present in 10.9% of samples | 32 |
| | PNRC1 | proline rich nuclear receptor coactivator | mutations present in 7.8% of samples | 32 |
| | SETD2 | histone methyltransferase | mutations present in 12.5% of samples | 32 |
| | PSMA1 | proteasome subunit | mutations present in 7.8% of samples | 32 |
| | MITF | melanogenesis associated transcription factor | mutations present in 3.1% of samples | 32 |
| | MYC | proto-oncogene | mutations present in 3.1% of samples | 32 |

| | GRIFIN | Galectin-related interfiber protein which binds carbohydrates | mutations present in 3.1% of samples | 32 |
|---|---|---|---|---|
| | ENSCAFG00000 031638 | uncharacterized protein | mutations present in 12.5% of samples | 32 |
| | FKBP3 | immunophilin that binds the immunosuppressants FK506 and rapamycin | mutations present in 4.7% of samples | 32 |
| | TBC1D26 | uncharacterized protein, may act as GTPase activator | mutations present in 6.3% of samples | 32 |
| | RPL23A | ribosomal subunit, may be involved in mediating growth inhibition | mutations present in 6.3% of samples | 32 |
| | SOCS2 | encodes cytokine signaling suppressor | mutations present in 4.7% of samples | 32 |
| | IGL | immunoglobulin lambda locus | frequently deleted, but deletions most likely not cancer-driven | 32 |
| | KLRK1 | killer cell lectin-like receptor subfamily K | deletions present in both B- and T-cell lymphoma, but significantly mutated only in B-cell lymphoma | 32 |
| | PKD1 | polycystin protein, an integral membrane protein important in cell-cell/matrix interactions | deletions present in both B and T cell lymphoma, but significantly mutated only in B-cell lymphoma | 32 |
| T cell (boxer) | PTEN | tumor suppressor | mutations present in 25% of samples | 32 |
| | SATB1 | SATB homeobox, regulates chromatin state and gene expression | mutations present in 25% of samples | 32 |
| | TBC1D26 | uncharacterized protein, may act as GTPase activator | mutations present in 6.3% of samples | 32 |
| | NLRP14 | innate immunity | mutations present in 12.5% of samples | 32 |
| | MAP2K1 | MAP kinase involved in proliferation, differentiation, transcription regulation and development | mutations present in 12.5% of samples | 32 |

| | KCND2 | voltage-gated potassium channel | mutations present in 12.5% of samples | 32 |
|---|---|---|---|---|
| | PSMA1 | proteasome subunit | mutations present in 6.3% of samples | 32 |
| | ENSCAFG00000031638 | uncharacterized protein | mutations present in 6.3% of samples | 32 |
| | TCR | T-cell receptor | frequently deleted, deletions are not cancer driven and most likely reflect original tumor cell | 32 |
| T cell (golden retriever) | PSMA1 | proteasome subunit | mutations present in 16% of samples | 32 |
| | COX8A | cytochrome c oxidase subunit involved in apoptosis | mutations present in 12% of samples | 32 |
| | LTA4H | acts as aminopeptidase | mutations present in 16% of samples | 32 |
| | NLRP5 | innate immunity | mutations present in 12% of samples | 32 |
| | SATB1 | SATB homeobox, regulates chromatin state and gene expression | mutations present in 12% of samples | 32 |
| | TBC1D26 | uncharacterized protein, may act as GTPase activator | mutations present in 20% of samples | 32 |
| | ZNF706 | transcription repressor | mutations present in 8% of samples | 32 |
| | ATP5H | ATP synthase | mutations present in 8% of samples | 32 |
| | ENSCAFG00000031638 | uncharacterized protein | mutations present in 12% of samples | 32 |
| | PTPN6 | protein tyrosine phosphatase signaling molecule | mutations present in 12% of samples | 32 |
| | GLUD2 | recycles glutamate during neurotransmission | mutations present in 12% of samples | 32 |

| RPL11 | ribosomal subunit | mutations present in 8% of samples | 32 |
|---|---|---|---|
| RPL23A | ribosomal subunit, may be involved in mediating growth inhibition | mutations present in 8% of samples | 32 |
| KRTAP10-6 | keratin associated protein | mutations present in 12% of samples | 32 |
| EEF1A1 | promotes binding of aminoacyl-tRNA to A site of ribosomes | mutations present in 12% of samples | 32 |
| MAGEC2 | enhance ubiquitin ligase activity of RING-type zinc finger-containing E3 ubiquitin-protein ligases | mutations present in 8% of samples | 32 |
| TCR | T-cell receptor | frequently deleted, deletions are not cancer driven and most likely reflect original tumor cell | 32 |
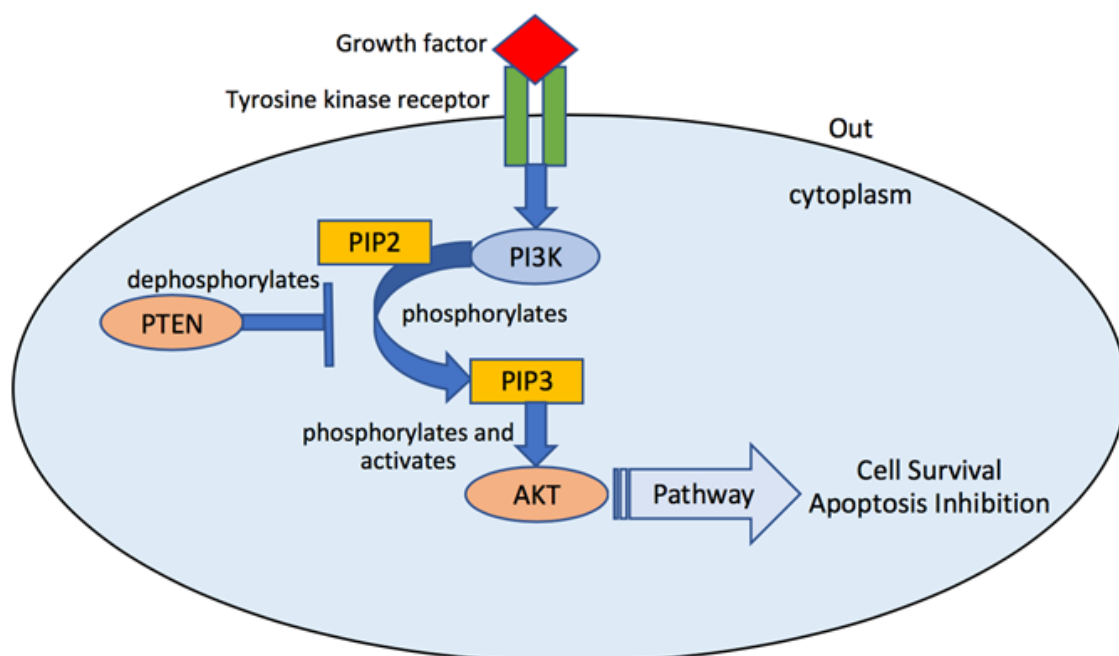


**Figure 1** PI3K–AKT–mTOR pathway. When a growth factor ligand binds to a receptor tyrosine kinase, it activates PI3K which phosphorylates PIP2 to become PIP3, which then activates AKT to cause cell survival and decreased apoptosis. PTEN antagonizes this pathway by dephosphorylating PIP3. Thus, mutations in PTEN cause cancer cell survival and proliferation. Reproduced from the open access source [37].

Another study done specifically on TRAF3 mutations in canine DLBCL found that 30% of 63 tumors contained at least one somatic TRAF3 mutation [35]. A majority of the mutations caused the loss of TRAF3 protein by affecting reading frame or creating a premature stop codon. The TRAF3 protein acts as a negative regulator in the NF-κB pathway, which is important in DNA transcription and cell survival, by targeting NF-κB-inducing-kinase for ubiquitination and degradation. With the constant underexpression of TRAF3, NF-κB-inducing-kinase levels remain high and constitutively activate the NF-κB pathway, which could easily play a role in the development of canine B-cell lymphomas, as well as many other cancers. Another study corroborated the reported underexpression of TRAF3, and found that the gene LIN28B, also involved in the NF-κB pathway, was the most frequently upregulated gene in tumors [38]. The study listed more differentially expressed genes that contributed to NF-κB overactivity, including CD79, CD19, SYK, LYN, CARD11, BCL10, BTK, TRAF6, MYD88, NFKB2, TLR7, and TLR9 [38].

Checkpoint molecule programmed cell death 1 (PD-1) protein was also examined for its use as a biomarker in canine lymphoma. PD-L1, the ligand of PD-1, was found to be overexpressed in malignant B-cells than normal B-cells, but normal and malignant T-cells showed low expression of both PD-1 and PD-L1 [40]. Furthermore, tumor infiltrating lymphocytes from both B and T cell lymphomas showed overexpression of PD-1 and PD-L1 compared to lymphocytes from healthy animals, demonstrating upregulated checkpoint molecule expression in lymphomas [40].

Due to the complexity of lymphoma subtype classification, it is difficult to trace specific genetic mutations or biomarkers that signal the development of lymphoma, but researchers have identified genes of interest that should be pursued further.

## 3. Mast Cell Tumors

Mast cells, also known as mastocytes or labrocytes, are a type of white blood cell. They reside in connective tissues and are derived from bone marrow. Although they are most commonly known for releasing histamines that induce inflammation during an allergic response, they also function in defense against parasitic infestations, tissue repair, and angiogenesis.

Mast cell neoplasms are hematopoietic disorders characterized by uncontrolled expansion and accumulation of neoplastic mast cells in various organ systems [20, 41].

In the study of cutaneous tumors in Swiss dogs, the most common tumor types were found mast cell tumors (16.35%), lipomas (12.47%), hair follicle tumors (12.34%), histiocytomas (12.10%), soft tissue sarcomas (10.86%), and melanocytic tumors (8.63%) [42].

Mast cell tumors (MCTs) are one of the most common canine skin neoplasms, accounting for 7%–21% of all cutaneous tumors [43]. They most commonly occur in middle-aged to elderly dogs, with a mean age of onset of nine years old. The tumor can occur in any breed of dog, although boxers, terriers, bulldogs, Weimaraners and Labrador retrievers have higher incidence rates [43, 44]. In Swiss dogs, the highest tumor incidence was found in the giant schnauzer, the standard schnauzer, the Magyar vizsla, the Rhodesian ridgeback, the Nova Scotia duck tolling retriever, and the boxer. Mixed-breed dogs had an increased incidence rate compared to the average of all breeds [42].

Mast cell tumors are usually categorized according to histologic grade. Two grading systems currently exist: the Patnaik system, which assigns a grade according to the degree of differentiation of the tumor, where "I" is well-differentiated, "II" is intermediately-differentiated,

and "III" is poorly-differentiated, and the Kiupel system, which categorizes a tumor as low or high grade according to survival time [45]. However, neither system predicts metastasis. It worth to note that COX-2 overexpression is associated with decreased overall survival and higher grades of malignancy according to Patnaik and Kiupel grading systems [46].

The proto-oncogene c-KIT has been identified as the key factor in the tumorigenesis of canine mast cell tumors. c-KIT codes for the type III receptor tyrosine kinase KIT, a cytokine receptor on the surface of hematopoietic stem cells that binds to stem cell factor and normally plays a role in cell proliferation, survival, decreased apoptosis and adhesion [47]. Like a typical receptor tyrosine kinase, once the ligand binds to KIT, it dimerizes and phosphorylates itself, which in turn activates more signaling molecules to continue signal transduction. However, according to one study, mutations have been identified in exon 11 of the juxtamembrane domain of c-KIT that lead to a constitutively activated KIT receptor (Figure 2) [reproduced with permission from reference 48]. These mutations are primarily internal tandem duplications (ranging from 39–69 bp in size) and deletions (Table 3) [43, 47, 49, 50]. Because the mutations cause the receptor to be activated even without a bound ligand, the cell proliferates uncontrollably, resulting in a tumor. Although c-KIT mutations were only found in 15% of the 60 tumors examined, they may be present in 30–50% of higher-grade mast cell tumors, as all the c-KIT mutations identified in the study were found in grade 2 or 3 tumors [43, 51]. This suggests that c-KIT mutations are associated with higher grade MCTs and thus a higher frequency of recurrent cancer and death. This finding was corroborated by a later study, which analyzed tumors graded with both systems in order to detect ITDs. It found that detection of tumor ITDs is significantly associated with both higher grades in both the Patnaik and Kiupel system [52].
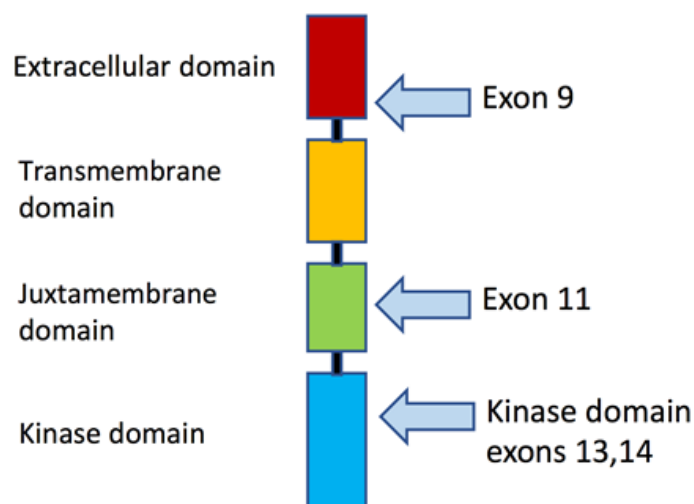


**Figure 2** Mutations were identified in exon 11 of *c-KIT*, which codes for the juxtamembrane portion of KIT. Reproduced from the open access source [48].

**Table 3** Mutations in *c-KIT* in canine mast cell tumors.

| Exon | Codon | Mutation | References |
|------|-------|----------|------------|
| 8 | 417-421 | internal tandem duplication (ITD) | 49 |
| 8 | 421-430 | Del421-430 InsLTFM | 50 |
| 8 | 430 | Q430R | 49 |
| 8 | 442 | G442D | 49 |
| 9 | 479 | S479I | 49 |
| 9 | 508 | N508I | 49 |
| 11 | 555-557 | Del555-557 InsV | 49 |
| 11 | 556-557 | Del556-557 | 49 |
| 11 | 557 | K557 InsF | 49 |
| 11 | 557 | K557N InsP | 49 |
| 11 | 557 | K557R Del558-559 | 49 |
| 11 | 571-579 | ITD | 49 |
| 11 | 571-581 | ITD | 49 |
| 11 | 571-582 | ITD | 50 |
| 11 | 571-583 | ITD | 49 |
| 11 | 571-585 | ITD | 49 |
| 11 | 571-589 | ITD | 49 |
| 11 | 572-583 | ITD | 49 |
| 11 | 572-585 | ITD | 49 |
| 11 | 572-586 | ITD | 49 |
| 11 | 572-587 | ITD | 49 |
| 11 | 572-588 | ITD | 49 |
| 11 | 572-589 | ITD | 49 |
| 11 | 572-590 | ITD | 49 |
| 11 | 573-585 | ITD | 49 |
| 11 | 573-590 | ITD | 49 |
| 11 | 573-591 | ITD | 49 |
| 11 | 574-587 | ITD | 50 |

| 11 | 575-582 | ITD | 49 |
| 11 | 576-590 | ITD | 49 |
| 17 | 826-828 | Del826-828 InsDT | 49 |

Canine MCTs with c-KIT mutations are also associated with aberrant protein localization to the cytoplasm [43]. Canine MCTs with a more cytoplasmic KIT localization are in turn more significantly associated with severe prognosis than MCTs with perimembrane KIT localization because the aberrant localization disrupts the signaling pathway. However, level of KIT expression does not correlate with c-KIT mutations or abnormal protein localization, suggesting that overexpression of KIT and the resulting increase in receptor sensitivity to the ligand are less influential than the constantly activated receptor and disruption of signaling pathways in the pathogenesis of mast cell tumors [43].

Although c-KIT mutations seem to be the primary contributor to mast cell tumors, other genes have been observed to contribute to a more severe prognosis and shorter survival times. Specific gene losses in PTEN, FAS, and CFA26 and gene gains in MAPK3, WNT5B, FGF, FOXM1, RAD51 and CFA27 are several potential candidate genes that can be examined for their effects on mast cell tumorigenesis [45, 53].

## 4. Osteosarcoma

Osteosarcoma (OSA) accounts for 85%–89% of all canine bone tumors [54, 55] and is the most common malignant bone tumor, typically affecting larger and older male dogs. The median age at diagnosis is eight years [13]. In particular, German shepherds, Labradors, Irish setters, Great Danes, Irish wolfhounds, Rottweilers and greyhounds are predisposed to the disease, and there seem to be certain breed-related risk factors present in addition to size-related risk factors [56, 57]. Osteosarcoma can also be categorized into two groups depending on its anatomical location. Appendicular osteosarcoma affects the limbs and is the more common type (75%), while axial osteosarcoma (24%) affects the skull, ribs, vertebrae and pelvis and is more common in smaller dogs [54]. Occasionally, it can affect soft tissues (1%) [54]. In addition to anatomical subtypes, osteosarcoma can also be divided into histologic subtypes, the most common three being osteoblastic, where tumor cells overproduce tumor osteoid; chondroblastic, where tumor cells produce chondroid and osteoid; and fibroblastic, where tumor cells are predominantly fibroblasts and produce both collagen and tumor osteoid. Two other less common subtypes include telangiectatic and giant cell type osteosarcoma [13, 58]. The target cell for malignant transformation of osteosarcoma is thought to be a mesenchymal stem cell or other cell committed to differentiating into an osteoblast [13]. A common symptom of osteosarcoma is limping or lameness in a limb with unknown cause [57]. Early detection of osteosarcoma is especially important because it may metastasize to the lungs.

A study conducted on greyhounds, Irish wolfhounds and Rottweilers identified 34 genetic loci involved in canine osteosarcoma tumorigenesis, among which somatic mutations were common in tumor suppressor genes RB1 and TP53, as well as CDK4 inhibitors CDKN2A/B (Table 4) [56, 58]. The most commonly mutated region was 150 kb upstream of the CDKN2A/B gene at chr11:44405676. This mutation would alter regulation of CDKN2A/ARF which encodes the INK4

family of cyclin-dependent kinase inhibitor proteins. The mutation would thus prevent the induction of senescence by the RB and p53 pathways. Other mutated genes include SEMA4D, SEMA6D, NF1, NF2 and PTEN [56]. More evidence suggests the importance of PTEN in osteosarcoma development—PTEN loss, either through deletion of CFA 26q25 (which encompasses the PTEN locus) or inactivating amino acid substitutions, was the most common aberration and occurred in 16 out 38 OSA cases studied [59, 61]. Other genomic losses include regions of CFA 16, 18, 29, and 35, especially the loss of gene WT1 at CFA 18q22.3 in 14 out of 38 cases [61]. Genomic gains of CFA 13q14 and CFA 31q15.3 were present in 16 out of 38 cases. This finding is consistent with the results of study on canine lymphoma stating that gain of CFA13 and CFA31 is an important step in tumorigenesis and is not specific to any form of cancer [56, 61].

**Table 4** Mutations and expression for canine osteosarcoma.

| Gene | Function | Mutation / Expression | References |
|------|----------|----------------------|------------|
| RB1 | encodes negative regulator of the cell cycle, stabilizes heterochromatin | recurrent point mutations observed | 56 |
| TP53 | tumor suppressor | mutated in exons 4–8 | 56 |
| CDKN2A/B | codes for two protein suppressors, control G1-progression by inactivation of D-cyclins | chr11:44405676 alters regulation of CDKN2A/ARF | 56 |
| SEMA4D | cell surface receptor important in cell-cell signaling, oncogene | overexpressed | 13 |
| SEMA6D | oncogene | overexpressed | 13 |
| NF1 | tumor suppressor | deletion or underexpression | 13 |
| NF2 | tumor suppressor | deletion or underexpression | 13 |
| PTEN | tumor suppressor | large deletions or potentially inactivating substitution at codon 340, nucleotide 1126 (A->T), asparagine -> tyrosine, resulting in underexpression | 59 |
| CTNNB1 | regulates cell–cell adhesion and gene expression, binds to Tcf and Lef transcription factors to upregulate expression of target genes such as c-myc, cyclin D1, survivin and matrix metalloproteinases | expressed in neoplastic cells but no mutations detected in exon 3 | 60 |

In another study examining p53 mutations, 19 out of 47 dogs with appendicular osteosarcoma and 5 out of 11 dogs with axial osteosarcoma were found to have p53 mutations (Table 5) [62]. Most of the 27 mutations observed were located on exons 4 and 5. Nineteen point-mutations observed resulted in an amino acid substitution, and the remaining seven mutations were deletions [62]. A majority of the point mutations were transitions between G:C->A:T with the second most common being transversions between G:C->T:A. Another study specifically sequenced exons 5–8 of p53 and found mutations in 47% of samples (Table 5) [63]. Most of the mutations were missense mutations in the highly conserved regions of p53, with the majority of them again being G: C->A: T transitions [63]. SETD2 was found to be the second most recurrently mutated gene in canine osteosarcoma after p53 [64]. SETD2 is a recognized tumor suppressor gene in human cancers but was not previously implicated in osteosarcoma. The study found SETD2 mutations in 21% of tumors across all three breeds of predisposed dogs (golden retrievers, Rottweilers and greyhounds).

**Table 5** TP53 and SETD2 aberrations data for canine osteosarcoma.

| Gene | Codon | Gene Aberrations | References |
|------|-------|------------------|------------|
| p53 | 7 | D7fs | 64 |
| p53 | 23 | W23* | 64 |
| p53 | 33 | S33fs | 64 |
| p53 | 34 | S34X | 62 |
| p53 | 50 | V50M | 62 |
| p53 | 52 | W52R | 62 |
| p53 | 52 | W52* | 64 |
| p53 | 78 | W78* | 64 |
| p53 | 91 | Q91fs | 64 |
| p53 | 92 | G92V | 64 |
| p53 | 92 | G92S | 64 |
| p53 | 106 | A106G | 64 |
| p53 | 107 | K107R | 62 |
| p53 | 107 | K107R | 64 |
| p53 | 112 | T112K | 64 |
| p53 | 112 | T112M | 64 |
| p53 | 122 | M122fs | 64 |
| p53 | 125 | A125V | 64 |
| p53 | 135 | S135G | 62 |
| p53 | 144 | R144H | 62 |
| p53 | 155 | F155Y | 62 |

| | | | |
|---|---|---|---|
| p53 | 160 | V160L | 64 |
| p53 | 160 | V160M | 64 |
| p53 | 161 | R161fs | 62 |
| p53 | 161 | R161W | 64 |
| p53 | 162 | R162H | 64 |
| p53 | 163 | C163F | 62 |
| p53 | 166 | H166R | 64 |
| p53 | 167 | E167K | 64 |
| p53 | 175 | G175_splice | 64 |
| p53 | 178 | P178L | 62 |
| p53 | 184 | R184* | 64 |
| p53 | 188 | L188I | 63 |
| p53 | 198 | N198fs | 64 |
| p53 | 202 | R202fs | 62 |
| p53 | 202 | H202R | 62 |
| p53 | 208 | Y208* | 64 |
| p53 | 220 | Y220S | 63 |
| p53 | 224 | Y224C | 64 |
| p53 | 226 | C226Y | 62 |
| p53 | 229 | S229fs | 64 |
| p53 | 237 | R237L | 64 |
| p53 | 248 | R248Q | 63 |
| p53 | 249 | R249W | 63 |
| p53 | 249 | S249_splice | 64 |
| p53 | 255 | R255P | 64 |
| p53 | 258 | F258S | 62 |
| p53 | 258 | E258K | 63 |
| p53 | 260 | V260I | 64 |
| p53 | 261 | R261Q | 62 |
| p53 | 261 | R261C | 64 |
| p53 | 261 | R261H | 64 |
| p53 | 261 | R261S | 64 |
| p53 | 262 | V262L | 64 |
| p53 | 266 | P266R | 62 |
| p53 | 266 | P266S | 64 |

| | | | |
|---|---|---|---|
| p53 | 276 | A276D | 63 |
| p53 | 282 | E282fs | 64 |
| p53 | 308 | K308X | 62 |
| p53 | 322 | G322W | 64 |
| p53 | 119-120 | Del119-120 | 62 |
| p53 | 143 | T143fs | 62 |
| p53 | 278-280 | Del278-280 InsR | 62 |
| SETD2 | 452 | R452* | 64 |
| SETD2 | 537 | R537* | 64 |
| SETD2 | 719 | Q719* | 64 |
| SETD2 | 1052 | D1052Y | 64 |
| SETD2 | 1403 | K1403fs | 64 |
| SETD2 | 1403 | K1403* | 64 |
| SETD2 | 1409 | I1409fs | 64 |
| SETD2 | 1457 | R1457* | 64 |
| SETD2 | 1470 | Y1470fs | 64 |
| SETD2 | 1570 | S1570_splice | 64 |
| SETD2 | 1801 | T1801fs | 64 |
| SETD2 | 1995 | E1995fs | 64 |
| SETD2 | 2096 | R2096_splice | 64 |
| SETD2 | 2377 | P2377fs | 64 |
| SETD2 | 2438 | G2438fs | 64 |
| SETD2 | 2542 | I2542fs | 64 |

The gene DLG2, important in regulating cell division, migration and tumorigenesis, is also a viable tumor suppressor candidate of osteosarcoma [65]. One study found that DLG2 copy number loss occurs in 56% of canine osteosarcomas. Deleting the DLG2 gene in a murine model also accelerated the development of canine osteosarcoma [65].

Other studies examined vulnerable pathways in osteosarcoma development in order to identify target genes. Transforming growth factor beta (TGFβ) and Hippo pathway mediators have important roles in bone development and cancer progression, but their importance in canine osteosarcoma has only recently been evaluated [66]. A study examined the role of Hippo signaling effectors TAZ and YAP, a transcriptional activator of genes involved in cell proliferation and apoptosis, along with pSmad2, a marker of active TGFβ signaling. It found that underexpression of both YAP and pSmad2 led to a slower metastasis [66]. This implies that inhibiting YAP and TAZ function and further studying the relationship between TGFβ and the Hippo pathway could prevent the spread and further development of osteosarcoma.

Wnt signaling pathway also plays a critical role in osteosarcoma disease progression because it controls the expression of beta-catenin, a transcriptional co-activator of many key proto-oncogenes such as MYC [67] (Figure 3) [reproduced from reference 67]. Beta-catenin expression levels are found to be frequently altered in osteosarcoma and beta-catenin is highly present in neoplastic cells. However, no mutations were identified in exon 3, suggesting that other regulatory mechanisms may play a larger role in beta-catenin accumulation [60].
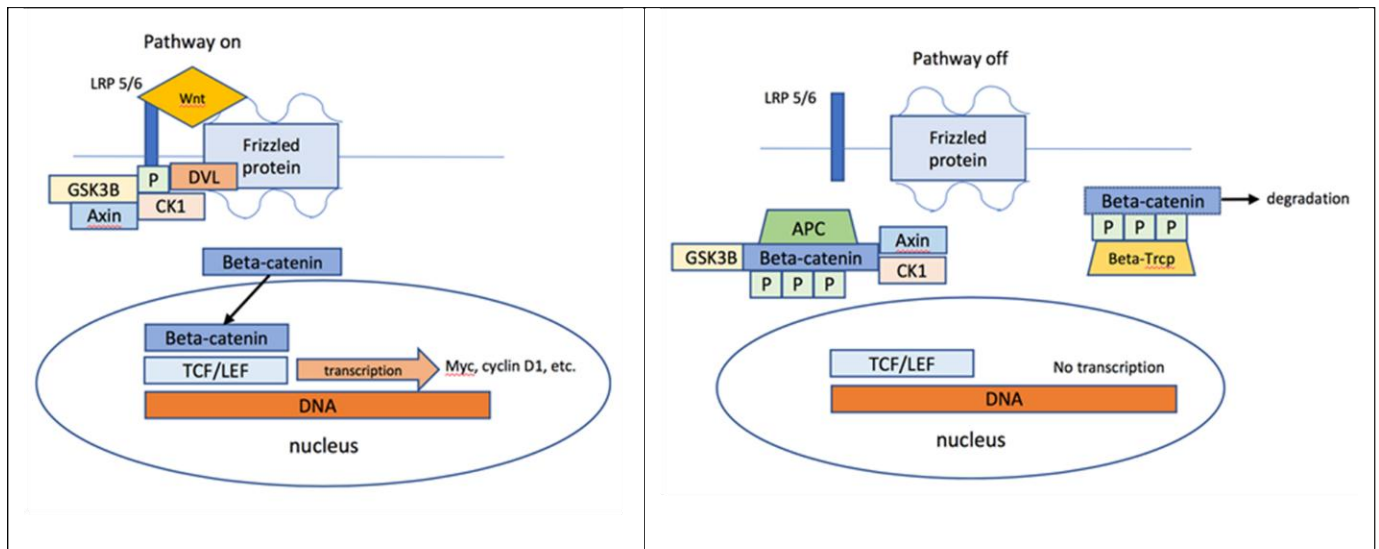


**Figure 3** Wnt signaling pathway. When Wnt binds to a frizzled protein, the Wnt pathway is activated and beta-catenin is able to facilitate the transcription of key genes such as proto-oncogene myc. When the Wnt pathway is off, beta-catenin is phosphorylated and tagged for destruction by beta Trcp. Reproduced from the open access source [67].

Another study found the genes MFAP4, CHRDL1, LOC100684002, and TMSB4X to be differentially expressed in aggressive tumors than non-aggressive tumors [68]. GDNF, CEMIP (KIAA1199), GDF6, ALPK2, GREM1, and DHRS2 were specifically underexpressed [68] These results were surprising because CEMIP is a target gene of the Wnt/β-catenin signaling pathway, which is overactive, and is known to promote cancer cell migration. The study also profiled osteosarcoma across species in order to identify common biomarkers of osteosarcoma. They identified ARK5, a serine-threonine protein kinase that regulates cellular senescence, as a new metabolic target present in all species and confirmed glucose metabolism as the most significantly aberrant cellular signaling pathway in metastatic tumors [68].

There are a wide variety of genes implicated in the development of osteosarcoma, and many show promises in targeted gene therapies specifically for treating osteosarcoma.

## 5. Mammary Gland Tumors

Mammary tumors make up more than 50% of the neoplasms in female dogs, making it the second most common neoplasia among dogs in general [69]. Miniature Poodles, Dachshunds, Malteses, Yorkshire Terriers, Cocker Spaniels and German Shepherds are the most predisposed

breeds [69]. Because the risk of developing mammary tumors is correlated with exposure to estrogen and progesterone from an early age, the risk of developing it increases if the dog is not spayed or is spayed after the age of two [70]. Approximately half of all mammary tumors in dogs are malignant, and tumors most often develop in the fourth and fifth mammary glands [70]. The most common type of malignant mammary tumor is tubular carcinoma (adenocarcinoma), followed by papillary carcinoma [69].

A comprehensive study done on expression levels of important cellular pathways in canine breast cancer found that cyclin-dependent kinase inhibitors, which regulate the cell cycle, are often dysfunctional. As expected, oncogenic pathways, such as PI3K/AKT, KRAS, MAPK, Wnt, β-catenin, BRCA2, ESR1 and P-cadherin, are upregulated while tumor suppressor pathways, like p53, p16/INK4A (encoded by CDKN2A), PTEN and E-cadherin, are downregulated (Table 6) [71]. Mutations in any of the genes or proteins involved in these pathways can lead to failure to check important cell events before continuing the cell cycle, which can lead to tumorigenesis. In particular, the p16/INK4A locus has been found to be frequently mutated. A study examining somatic copy number alterations corroborated these findings, revealing that the oncogene c-MYC was the most recurrently amplified region, and the tumor suppressor PTEN was frequently lost. The study also pointed out genes COL9A3, INPP5A, CYP2E1 and RB1 as other possible contributors to breast cancer tumorigenesis [77].

**Table 6** Expression and aberrations of genes in mammary gland tumors.

| Gene | Function | Expression or/and Aberration | References |
|---|---|---|---|
| BRCA2 | homologous recombination repair, suppress tumorigenesis, proto-oncogenic | less expressed in tumors, single nucleotide variations in exon 11, frame shift leading to nonsense mediated mRNA decay and variations at 2 hotspots | 72 |
| TP53 | tumor suppressor | downregulated, G:C → T:A (17%) and A:T → T:A (17%) transversions and G:C → A:T (67%) transitions (total 20% frequency in exons 5-8) | 71, 73 |
| AKT1 | proto-oncogenic | upregulated | 71 |
| PI3K | proto-oncogenic | upregulated | 71 |
| KRAS | proto-oncogenic | upregulated | 71 |
| MAPK | proto-oncogenic | upregulated | 71 |
| CTNNB1 | controls cell-cell adhesion and gene expression, proto-oncogenic | upregulated | 71 |
| ESR1 | proto-oncogenic | upregulated | 71 |

| CDH3 | proto-oncogenic | upregulated | 71 |
|------|-----------------|-------------|----|
| p16/INK 4a | tumor suppressor | downregulated | 71 |
| PTEN | reduces cell proliferation, involved in apoptosis and cell adhesion | downregulated | 71 |
| CDH1 | tumor suppressor | downregulated | 71 |
| COX2 | conversion of arachidonic acid to prostaglandin H2 | increased expression associated with increasing malignancy | 74–76 |
| ERBB2 | proto-oncogene | loss of ERBB2 expression has been associated with a poor prognosis when linked with estrogen receptor negativity and expression of one of 3 basal cell markers (P-cadherin, p63, cytokeratin 5) | 76 |

BRCA2 is an important tumor suppressor in both humans and canines. It binds to Rad51 recombinase through interaction with eight BRC repeats, and the complex repairs DNA damage through homologous recombination repair (Figure 4) [72, 78-80]. In one study, it was found that mammary tumors express less BRCA2 than normal mammary glands, which would explain how mutations causing breast cancer might arise [72]. However, the cause for the underexpression is unclear—there were no mutations in the promoter that might affect transcription levels. The study discovered two BRCA2 splice variants, one of which induced a shift in reading frame that lead to nonsense-mediated RNA decay and thus underexpression [72]. In addition, there were many single nucleotide polymorphisms in exon 11 of BRCA2 (Table 7) and a high frequency of genetic variation at two "hot spots" (A511C and A2414G) in many tumors that could have led to BRCA2 underexpression [79, 81, 82]. Two key mutations were also found in BRC repeat 3, a substitution from Threonine to Proline at codon 1425 and Lysine to Arginine at codon 1435, that would affect the affinity between BRC3 and Rad51 and increase the risk of developing breast cancer [78]. The discovery of BRCA2 underexpression in this study contrasts with other studies who report no difference in BRCA2 expression levels between benign and malignant tumors [69]. Thus, BRCA2 plays an important role in the development of breast cancer, but the exact link between BRCA2 expression level and breast cancer tumorigenesis is still unclear.
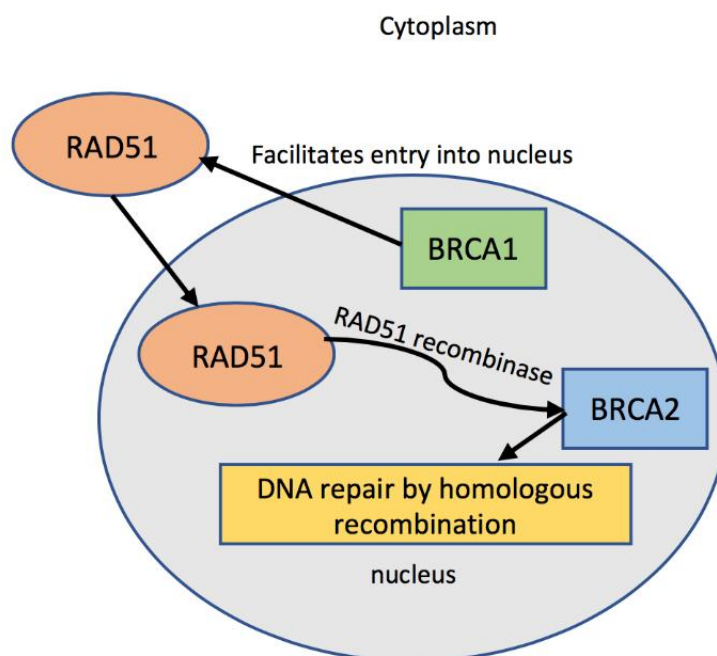
**Figure 4** Interaction between BRCA2 and Rad51 in homologous recombination DNA repair (all related to reproduction) Reproduced from the open access source [80].

**Table 7** Amino-acid substitution mutations in canine mammary tumors.

| Gene | Exon | Codon | Amino Acid Substitution | References |
|------|------|-------|-------------------------|------------|
| p53 | 7 | 249 | R249W | 68 |
| p53 | 5 | 148 | S148R | 68 |
| p53 | 8 | 271 | E271V | 68 |
| p53 | 5 | 175 | H175A | 68 |
| p53 | 5 | 163 | I163F | 68 |
| p53 | 7 | 245 | G245D | 68 |
| p53 | 7 | 252 | L252F | 68 |
| p53 | 5 | 180 | E180X | 68 |
| p53 | 7 | 245 | G245A | 64 |
| p53 | 5 | 173 | V173L | 64 |
| p53 | 8 | 285 | P285S | 64 |
| p53 | 5 | 129 | L129F | 64 |
| p53 | 7 | 248 | R248Q | 64 |
| p53 | 8 | 297 | P297R | 64 |
| p53 | 6 | 213 | R213X | 64 |
| BRCA2 | 11 | 669 | N669D | 64 |
| BRCA2 | 11 | 801 | K801Q | 64 |
| BRCA2 | 11 | 908 | E908Q | 62 |
| BRCA2 | 11 | 1425 | T1425P | 61 |
| BRCA2 | 11 | 1435 | K1435R | 62 |

The most frequently studied biomarker of mammary tumors is Ki-67, a nuclear, non-histone protein, which can only be detected in the cell nucleus during interphase, and during mitosis. Higher levels of Ki-67 are associated with increased chances for metastasis and poorer prognosis [69, 83, 84]. Another biomarker is PCNA, an auxiliary protein of DNA polymerase δ, involved in the DNA repair process, cell cycle control, chromatin assembly and in RNA transcription [69, 83]. PCNA expression level has a positive correlation with tumor size, histological grade of malignancy, and lymph node metastasis. Carvalho and colleagues had shown that PI of intratumoral Ki-67 and PCNA are statistically associated with the following tumor characteristics: higher aggressiveness, tumor histological grade, nuclear grade, and lymph node involvement [85]. In general, high intratumoral Ki-67 and PCNA are associated with shorter survival [85]. However, since PCNA is stimulated by cytokines, it may not be solely indicative of cancer. Thus, PCNA presence should be evaluated in conjunction with other biomarkers.

A further look at the important tumor suppressor and biomarker p53 again revealed that p53 mutations in the highly conserved domains of exons 5–8 are correlated to tumorigenesis and increased malignancy (Table 7) [69, 73, 81, 83]. p53 is thought to be the most frequently mutated gene in mammary tumors [69]. In total, 20% of the 25 mammary tumors studied had mutations in exons 5–8, a frequency which is similar to that in human breast cancer. Just as in canine osteosarcoma and many other cancers, a majority of the mutations were G:C->A:T transitions, with the next most common being G:C->T:A and T:A->A:T transversions [73].

Another gene presented as a promising tumor suppressor in canine breast cancer is EZH2, which codes for a catalytic subunit of a complex that leads to silencing of genes involved in processes such as stem cell maintenance and tumor progression without DNA sequence modification. It was found to be overexpressed in canine breast cancer and human breast cancer [86].

Many biomarkers have already been discovered that show promise in clarifying the mechanisms behind breast cancer. However, the links between them and tumorigenesis still remain unclear, so markers such as BRCA2, Ki-67, PCNA, and p53 cannot be evaluated individually in order to determine the development of breast cancer.

## 6. Melanoma

As the fourth most common cancer in dogs overall, melanoma represents 4%–7% of all cancers and 9%–20% of skin neoplasms [87]. Melanomas are classified based on their location and are generally categorized as cutaneous, ocular, oral or subungual (on the nail bed) melanoma. The primary tumor site is the oral cavity (56%); other less common sites include the lips (23%), skin (11%), eyes (3%) and digits (8%) [23, 88]. Older dogs (an average age of nine years) are more predisposed to the disease, and there is no gender influenced selection, although males are typically overrepresented [89]. Purebred dogs, breeds such as poodles, dachshunds, golden retrievers, schnauzers, cocker spaniels, and Scottish terriers, and dogs with darker pigmented skin are more at risk of developing melanoma [23, 87, 89]. This marks a surprising difference between humans and dogs, as humans with lighter skin and less melanin (which protects from UV rays), are generally more at risk for melanoma. This leads to the hypothesis that UV radiation is not a significant factor in the genesis of canine melanoma, possibly because of the protective hair coat [15] (Table 8) [adapted from reference 23].

**Table 8** Common sites of malignant melanomas in dogs.

| Sites / Total Number of Cases | 2004 cases | Percentage |
|---|---|---|
| Oral | 1150 | 57.39 |
| Cutaneous | 580 | 28.94 |
| Scrotum | 4 | 0.20 |
| Digit | 115 | 5.74 |
| Ungual | 60 | 2.99 |
| Ocular | 41 | 2.05 |
| Lips/Feet | 54 | 2.69 |
| **References** | 89-91 | |

Genes implicated in human melanoma include important proto-oncogenes and tumor suppressors BRAF, NRAS, PTEN, KIT, GNAQ, and CDK4. Of those six, mutations were only observed in NRAS and PTEN in canine melanoma and were in locations corresponding to the human mutations (Table 9) [89]. Most importantly, canines lack key mutations in BRAF exon 15 that lead to the development of human melanomas [89]. Mutations in BRAF exon 15 are associated with skin exposure to UV light; mutations in human BRAF lead to PI3K/AKT/mTOR signaling pathway activation [15, 89, 92]. Yet despite the lack of activating BRAF mutations in canines, the mitogen-activated protein kinase (MAPK) and/or phosphoinositide 3-kinase (PI3K) pathways are still activated in 52%–77% of cases [15]. This may be due to PTEN and NRAS loss, or overexpression of receptor tyrosine kinases, such as platelet derived growth factor receptor (PDGFR). Another gene that may play a role in activated MAPK signaling is IQGAP1, which regulates oncogenic ERK1/2 MAPK signaling [93]. IQGAP1 expression is increased in tumors, especially co-localizing with melanocytes as well as at the tumor edge [93]. Targeted gene therapy focusing on the interaction between IQGAP1 and ERK1/2 has already started and holds promise.

**Table 9** Mutations in canine oral melanoma tumors.

| Genes | Exon | Codon | Amino Acid Substitution | References |
|-------|------|-------|------------------------|------------|
| NRAS | 2 | 61 | Q61R | 89 |
| NRAS | 2 | 61 | Q61H | 89 |
| PTEN | 7 | 251 | G251C | 89 |
| PTEN | 7 | 252 | D252X | 89 |
| p53 | | 92 | Y92* | 15 |
| p53 | | 147 | R147C | 15 |
| p53 | | 153 | K153fs | 15 |
| p53 | | 272 | R272H | 15 |
| p53 | | 292 | G292R | 15 |
| p53 | | 308 | Q308* | 15 |
| PTPRJ | | 1 | M1fs | 95 |
| PTPRJ | | | 346+2_346+3insCATG | 95 |
| PTPRJ | | 208 | T208fs | 95 |
| PTPRJ | | 364 | A364G | 95 |
| PTPRJ | | 634 | F634fs | 95 |
| PTPRJ | | 982 | A982fs | 95 |
| PTPRJ | | 1015 | K1015* | 95 |
| PTPRJ | | 1098 | D1098fs | 95 |
| PTPRJ | | | Asn1102_Lys1112delinsLys | 95 |

Beyond that, deletion of the genes encoding the tumor suppressors p53, Rb, p21 (waf-1), p16 (ink-4a), and PTEN have been postulated to contribute to the pathogenesis of melanoma [23] In particular, the loss of p16 and PTEN are common abnormalities observed in melanoma. Frequent MYC amplifications and deletions of CDKN2A were also observed [15]. Expression of cell–cell adhesion molecules was also altered, such as decreased expression of E-cadherin, V-CAM1, increased expression of N-cadherin, Mel-CAM1, ICAM 1, and α β integrins (Table 10) [23]. This is important because disturbing the interaction between melanocytes and basal keratinocytes may cause the melanocytes to divide uncontrollably, creating a malignant tumor that can then metastasize. Cell adhesion molecules also play an important role in tumor suppression because cell division is often inhibited when in contact with other cells.

**Table 10** Expressions and aberrations in canine melanoma.

| Gene | Function | Expression or/and Aberration | Reference |
|---|---|---|---|
| TP53 | tumor suppressor— cell cycle control and DNA repair | expression undetectable or mutations present | 87 |
| RB-1 | cell cycle control | expression undetectable | 87 |
| p21/waf-1 | Pan-CDK inhibitor and stress activated protein kinase inhibitor | expression undetectable, deletions common in humans | 87 |
| p16/ink-4a | inhibits CDK4 and CDK6 | deletions common in many tumors | 87 |
| PTEN/MMAC-1 | lipid/tyrosine phosphatase, tumor suppressor | PTEN deleted on chromosome 10 | 87 |
| CDH1 | cell–cell adhesion, E cadherin | decreased expression | 23 |
| V-CAM1 | vascular cell adhesion | decreased expression | 23 |
| CDH2 | cell–cell adhesion, N cadherin | increased expression | 23 |
| MCAM | melanoma cell adhesion | increased expression | 23 |
| ICAM1 | intercellular adhesion | increased expression | 23 |
| ITGA1 | cell–cell adhesion, alpha integrin | increased expression | 23 |
| ITGB2 | cell–cell adhesion, beta integrin | increased expression | 23 |

Recently, a novel tumor suppressor gene PTPRJ was linked to the development of canine malignant melanoma [15]. PTPRJ is a protein tyrosine phosphatase receptor that has previously been linked to oncogenesis and plays a role in allelic loss and loss of heterozygosity in humans. The study found that 7 out of 37 sequenced tumors (19%) bore a total of 9 PTPRJ mutations, most of which were truncating mutations. The study sequenced six frameshift and nonsense mutations, one splice site mutation, one 10-amino-acid-long deletion, and one missense mutation [15].

Members of the PI3K/AKT/mTOR and ERK1/2 pathways show promise for targeted gene therapy of canine melanoma, as well as the gene PTPRJ, which has not previously been linked to canine cancers.

## 7. Conclusion

We live in era of rapidly developing new methods in medicine. Two main achievements of medicine— vaccination and antibiotics, saved millions of people and animals. Now we are entering a new era of personalized medicine. The next NG sequencing that can elucidate driver mutations for various cancers become a usual practice in human cancer medicine. Understanding the complex molecular mechanisms including consequent somatic mutation leading to tumor development is ushering in revolution of cancer treatments. Now we can address the discovered molecular cancerogenic aberration directly and propose the most optimal combinations of drug covering the majority of these. Study of molecular mechanisms of cancer development in animals is extremely important from the point of comparative medicine. As pointed by Kent Lloyd and colleagues, "molecular phenotyping of animal diseases will connect those conditions with similarly characterized human disorders for which precision treatments have been or are being developed" [94]. In the same time, we need to note that current "state of art" in canine cancer therapy that includes elucidation of very few cancer-related biomarkers cannot be a basis to the precision-medicine-targeted therapy solutions. From this point the works that include elucidation of the concrete aberrations related to specific canine cancers are hard to overestimate.

In this review we summarized more than 30 canine cancer-related molecular aberrations elucidated by various methods along with more than 30 expression markers of these cancers. Interesting to note that many of them are not on the list of most frequent aberrations in human cancers. These findings pave the road to serious changes in canine cancer medicine and we hope to revolution toward personalized cancer treatment for dogs and eventually to other animals.

## Author Contributions

IT suggested general outline of the article and selected the specified cancers for description. VK and JC wrote the article.

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## Competing Interests

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**References**

1. Gardner HL, Fenger JM, London CA. Dogs as a model for cancer. Annu Rev Anim Biosci. 2016; 4: 199-222.
2. Pang LY, Argyle DJ. Veterinary oncology: Biology, big data and precision medicine. Vet J. 2016; 213: 38-45.
3. Rowell JL, McCarthy DO, Alvarez CE. Dog models of naturally occurring cancer. Trends Mol Med. 2011; 17: 380-388.
4. Raposo TP, Arias-Pulido H, Chaher N, Fiering SN, Argyle DJ, Prada J, et al. Comparative aspects of canine and human inflammatory breast cancer. Semin Oncol. 2017; 44: 288-300.
5. Queiroga FL, Raposo T, Carvalho MI, Prada J, Pires I. Canine mammary tumours as a model to study human breast cancer: most recent findings. In Vivo. 2011; 25: 455-465.
6. Raposo TP, Pires I, Prada J, Queiroga FL, Argyle DJ. Exploring new biomarkers in the tumour microenvironment of canine inflammatory mammary tumours. Vet Comp Oncol. 2017; 15: 655-666.
7. Merlo DF, Rossi L, Pellegrino C, Ceppi M, Cardellino U, Capurro C, et al. Cancer incidence in pet dogs: Findings of the animal tumor registry of Genoa, Italy. J Vet Intern Med. 2008; 22: 976-984.
8. Seelig D, Avery A, Ehrhart E, Linden M. The Comparative diagnostic features of canine and human lymphoma. Vet Sci. 2016; 3: 11.
9. Moore AS, Frimberger AE. Usefulness of chemotherapy for the treatment of very elderly dogs with multicentric lymphoma. J Am Vet Med Assoc. 2018; 252: 852-859.
10. Fulkerson CM. Multicentric lymphoma in the dog: An overview of diagnostics, treatment and prognosis. Indiana: Veterinary Association; 2016. Available from: http://invma.org/wp-content/uploads/sites/5/2016/09/Lymphoma-in-the-Dog.pdf
11. Ernst T, Kessler M, Lautscham E, Willimzig L, Neiger R. Multicentric lymphoma in 411 dogs – An epidemiological study. Tierarztl Prax Ausg K Kleintiere Heimtiere. 2016; 44: 245-251.
12. Flory AB, Rassnick KM, Stokol T, Scrivani PV, Erb HN. Stage migration in dogs with lymphoma. J Vet Intern Med. 2007; 5: 1041-1047.
13. Varshney J, Scott M, Largaespada D, Subramanian S. Understanding the osteosarcoma pathobiology: A comparative oncology approach. Vet Sci. 2016; 3: E3.
14. Scott MC, Temiz NA, Sarver AE, LaRue RS, Rathe SK, Varshney J, et al. Comparative transcriptome analysis quantifies immune cell transcript levels, metastatic progression and survival in osteosarcoma. Cancer Res. 2018; 78: 326-337.
15. Hernandez B, Adissu HA, Wei BR, Michael HT, Merlino G, Simpson RM. Naturally occurring canine melanoma as a predictive comparative oncology model for human mucosal and other triple wild-type melanomas. Int J Mol Sci. 2018; 19: 394.
16. Lejeune A, Skorupski K, Frazier S, Vanhaezebrouck I, Rebhun RB, Reilly CM, et al. Aggressive local therapy combined with systemic chemotherapy provides long-term control in grade II stage 2 canine mast cell tumour: 21 cases (1999–2012). Vet Comp Oncol. 2015; 13: 267-280.
17. ACVIM Fact Sheet: Canine Lymphoma. ACVIM—American: College of Veterinary Internal Medicine; 2014 [2019/03/28]. Available from: http://www.acvim.org/Portals/0/PDF/Animal%20Owner%20Fact%20Sheets/Oncology/Canine%20Lymphoma.pdf
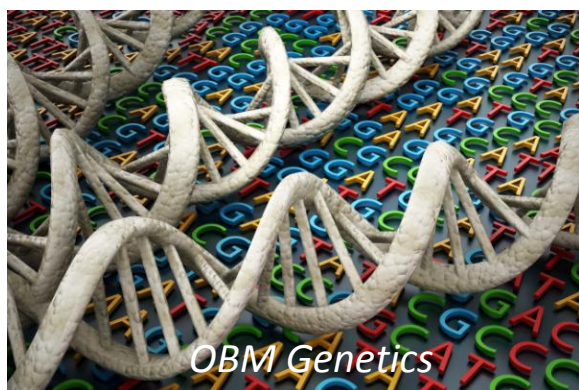
18. Ehrhart NP, Ryan SD, Fan TM. Tumors of the skeletal system. In: Withrow SJ, Vail DM, Page RP, editors. Withrow and MacEwen's Small Animal Clinical Oncology, 5th ed. St. Louis, Mo.: Esevier–Saunders, 2013. Chapter 2, pp. 463-503.

19. Selmic LE, Ryan SD, Ehrhart NP, Withrow SJ. Bilateral appendicular bone tumors in four dogs. J Am Anim Hosp Assoc. 2013; 49: 135-141.

20. Sledge DG, Webster J, Kiupel M. Canine cutaneous mast cell tumors: A combined clinical and pathologic approach to diagnosis, prognosis, and treatment selection. Vet J. 2016; 215: 43-54.

21. Warland, J, Brioschi, V, Owen, L, Dobson, J. Canine mast cell tumours: decision-making and treatment. In Pract. 2015; 37: 315-332.

22. London CA, Malpas PB, Wood-Follis SL, Boucher JF, Rusk AW, Rosenberg MP, et al. Multi-center, placebo-controlled, double-blind, randomized study of oral toceranib phosphate (SU11654), a receptor tyrosine kinase inhibitor, for the treatment of dogs with recurrent (either local or distant) mast cell tumor following surgical excision. Clin Cancer Res. 2009; 15: 3856-3865.

23. Nishiya AT, Massoco CO, Felizzola CR, Perlmann E, Batschinski K, Tedardi MV, et al. Comparative aspects of canine melanoma. Vet Sci. 2016; 3: E7.

24. Verganti S, Berlato D, Blackwood L, Amores-Fuster I, Polton GA, Elders R, et al. Use of oncept melanoma vaccine in 69 canine oral malignant melanomas in the UK. J Small Anim Pract. 2017; 58: 10-16

25. Nunes FC, Damasceno KA, de Campos CB, Bertagnolli AC, Lavalle GE,' Cassali GD. Mixed tumors of the canine mammary glands: Evaluation of prognostic factors, treatment, and overall survival. Vet Anim Sci. 2019; 7: 100039.

26. Sorenmo, K. Canine mammary gland tumors. Vet Clin North Am Small Anim Pract. 2003; 33: 573–596.

27. Sorenmo KU, Worley DR, Goldschmidt MH. Tumors of the mammary gland. In: Withrow SJ, Vail DM, Page RP, editors. Withrow and MacEwen's Small Animal Clinical Oncology, 5th ed. St. Louis, Mo.: Esevier–Saunders, 2013. Chapter 27, pp. 538-556.

28. Cassali GD, Lavalle GE, deNardi AB, Ferreira E, Bertagnolli AC, Estrela-Lima A, et al. Consensus for the diagnosis, prognosis and treatment of canine mammary tumors. Braz J Vet Pathol. 2011; 4: 153-180.

29. Lavalle GE, De Campos CB, Bertagnolli AC, Cassali GD. Canine malignant mammary gland neoplasms with advanced clinical staging treated with carboplatin and cyclooxygenase inhibitors. In Vivo. 2012; 26: 375-379.

30. Karayannopoulou M, Kaldrymidou E, Constantinidis TC, Dessiris A. Adjuvant post-operative chemotherapy in bitches with mammary cancer. J Vet Med A Physiol Pathol Clin Med. 2001; 48: 85-96.

31. Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M. Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. Br J Cancer. 2003; 89: 1530-1537.

32. Elvers I, Turner-Maier J, Swofford R, Koltookian M, Johnson J, Stewart C, et al. Exome sequencing of lymphomas from three dog breeds reveals somatic mutation patterns reflecting genetic background. Genome Res. 2015; 25: 1634-1645.

33. Zandvliet M. Canine lymphoma: A review. Vet Q. 2016; 36: 76-104.

34. Koshino A, Goto-Koshino Y, Setoguchi A, Ohno K, Tsujimoto H. Mutation of p53 gene and its correlation with the clinical outcome in dogs with lymphoma. J Vet Intern Med. 2016; 30: 223-229.

35. Bushell KR, Kim Y, Chan FC, Ben-Neriah S, Jenks A, Alcaide M, et al. Genetic inactivation of TRAF3 in canine and human B-cell lymphoma. Blood. 2015; 125: 999-1005.

36. Otto C, Giefing M, Massow A, Vater I, Gesk S, Schlesner M, et al. Genetic lesions of the TRAF3 and MAP3K14 genes in classical Hodgkin lymphoma. Br J Haematol. 2012; 157: 702-708.

37. Molinari F, Frattini M. Functions and regulation of the PTEN Gene in colorectal cancer. Front Oncol. 2014; 3: 326.

38. Aresu L, Ferraresso S, Marconato L, Cascione L, Napoli S, Gaudio E, et al. New molecular and therapeutic insights into canine diffuse large B cell lymphoma elucidates the role of the dog as a model for human disease. Haematologica. 2018; 103.

39. Richards KL, Motsinger-Reif AA, Chen HW, Fedoriw Y, Fan C, Nielsen DM, et al. Gene profiling of canine B-Cell lymphoma reveals germinal center and postgerminal center subtypes with different survival times, modeling human DLBCL. Cancer Res. 2013; 73: 5029-5039.

40. Hartley G, Elmslie R, Dow S, Guth A. Checkpoint molecule expression by B and T cell lymphomas in dogs. Vet Comp Oncol. 2018; 16: 352-360.

41. Willmann M, Hadzijusufovic E, Hermine O, Dacasto M, Marconato L, Bauer K, et al. Comparative oncology: The paradigmatic example of canine and human mast cell neoplasms. Vet Comp Oncol. 2019; 17: 1-10.

42. Graf R, Pospischil A, Guscetti F, Meier D, Welle M, Dettwiler M. Cutaneous tumors in swiss dogs: retrospective data from the swiss canine cancer registry, 2008-2013. Vet Pathol. 2018; 55: 809-820.

43. Webster JD, Yuzbasiyan-Gurkan V, Kaneene JB, Miller R, Resau JH, Kiupel M. The role of c-KIT in tumorigenesis: Evaluation in canine cutaneous mast cell tumors. Neoplasia. 2006; 8: 104-111.

44. Simoes JP, Schoning P, Butine M. Prognosis of canine mast cell tumors: A comparison of three methods. Vet Pathol. 1994; 31: 637-647.

45. Blacklock KB, Birand Z, Biasoli D, Fineberg E, Murphy S, Flack D, et al. Identification of molecular genetic contributants to canine cutaneous mast cell tumour metastasis by global gene expression analysis. PLoS One. 2018; 18: e0208026.

46. Gregório H, Raposo T, Queiroga FL, Pires I, Pena L, Prada J. High COX-2 expression in canine mast cell tumours is associated with proliferation, angiogenesis and decreased overall survival. Vet Comp Oncol. 2017; 15: 1382-1392.

47. Patruno R, Marech I, Zizzo N, Ammendola M, Nardulli P, Gadaleta C, et al. C-kit expression, angiogenesis, and grading in canine mast cell tumour: A unique model to study C-kit driven human malignancies. Biomed Res Int. 2014; 2014: 730246.

48. Maki R, Keedy V. KIT Exon 11 Mutation in GIST. My cancer genome; 2014 [2019/03/28]. Available from: https://www.mycancergenome.org/content/disease/gist/kit/47/.

49. Letard S, Yang Y, Hanssens K, Palmérini F, Leventhal PS, Guéry S, et al. Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. Mol Cancer Res. 2008; 6: 1137-1145.

50. Marconato L, Zorzan E, Giantin M, Di Palma S, Cancedda S, Dacasto M. Concordance of c-kit mutational status in matched primary and metastatic cutaneous canine mast cell tumors at baseline. J Vet Intern Med. 2014; 28: 547-553.

51. Zemke, D, Yamini, B, Yuzbasiyan-Gurkan, V. Mutations in the juxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. Vet Pathol. 2002; 39: 529-535.

52. Tamlin VS, Kessell AE, Mccoy RJ, Dobson EC, Smith TS, Hebart M, et al. Prevalence of exon 11 internal tandem duplications in the C-KIT proto-oncogene in Australian canine mast cell tumours. Aust Vet J. 2017; 10: 386-391.

53. Jark PC, Mundin DBP, de Carvalho M, Ferioli RB, Anai LA, Marchi FA, et al. Genomic copy number variation associated with clinical outcome in canine cutaneous mast cell tumors. Res Vet Sci. 2017; 111: 26-30.

54. Morello E, Martano M, Buracco P. Biology, diagnosis and treatment of canine appendicular osteosarcoma: Similarities and differences with human osteosarcoma. Vet J. 2011; 189: 268-277.

55. Liptak JM, Dernell, WS, Ehrhart, NP, Withrow, SJ. Canine appendicular osteosarcoma: diagnosis and palliative treatment. Compend Contin Educ Vet. 2004; 26: 172-182.

56. Karlsson, EK, Sigurdsson, S, Ivansson, E, Thomas, R, Elvers, I, Wright, J, et al. Genome-wide analyses implicate 33 loci in heritable dog osteosarcoma, including regulatory variants near CDKN2A/B. Genome Biol. 2013; 14: R132.

57. Sapierzyński, R, Czopowicz, M. The animal-dependent risk factors in canine osteosarcomas. Polish J Vet Sci 2017; 20: 293-298.

58. Simpson, S, Dunning, MD, de Brot, S, Grau-Roma, L, Mongan, NP, Rutland, CS. Comparative review of human and canine osteosarcoma: morphology, epidemiology, prognosis, treatment and genetics. Acta Vet Scand. 2017; 59: 71.

59. Levine, RA, Forest, T, Smith, C. Tumor suppressor PTEN is mutated in canine osteosarcoma cell lines and tumors. Vet Pathol. 2002; 39: 372-378.

60. Stein TJ, Holmes KE, Muthuswamy A, Thompson V, Huelsmeyer MK. Characterization of β-catenin expression in canine osteosarcoma. Vet Comp Oncol. 2011; 9: 65-73.

61. Thomas R, Wang HJ, Tsai PC, Langford CF, Fosmire SP, Jubala CM, et al. Influence of genetic background on tumor karyotypes: Evidence for breed-associated cytogenetic aberrations in canine appendicular osteosarcoma. Chromosome Res. 2009; 17, 365-377.

62. Kirpensteijn J, Kik M, Teske E, Rutteman GR. TP53 gene mutations in canine osteosarcoma. Vet Surg. 2008; 37: 454-460.

63. Johnson AS, Couto CG, Weghorst CM. Mutation of the p53 tumor suppressor gene in spontaneously occurring osteosarcomas of the dog. Carcinogenesis. 1998; 19: 213-217.

64. Sakthikumar S, Elvers I, Kim J, Arendt ML, Thomas R, Turner-Maier J, et al. SETD2 is recurrently mutated in whole-exome sequenced canine osteosarcoma. Cancer Res. 2018; 78: 3421-3431.

65. Shao YW, Wood GA, Lu J, Tang QL, Liu J, Molyneux S, et al. Cross-species genomics identifies DLG2 as a tumor suppressor in osteosarcoma. Oncogene 2019; 38: 291-298.

66. Luu AK, Schott CR, Jones R, Poon AC, Golding B, Hamed R, et al. An evaluation of TAZ and YAP crosstalk with TGFβ signalling in canine osteosarcoma suggests involvement of hippo signalling in disease progression. BMC Vet Res. 2018; 14: 365.

67. Lin CH, Ji T, Chen CM, Hoang BH. Wnt signaling in osteosarcoma. Adv Exp Med Biol. 2014; 804: 33-45.

68. Davis LE, Jeng S, Svalina MN, Huang E, Pittsenbarger J, Cantor EL, et al. Integration of genomic, transcriptomic and functional profiles of aggressive osteosarcomas across multiple species. Oncotarget. 2017; 8: 76241-76256.

69. Kaszak I, Ruszczak A, Kanafa S, Kacprzak K, Król M, Jurka P. Current biomarkers of canine mammary tumors. Acta Vet Scand. 2018; 60: 66.

70. McCourt MR, Dieterly AM, Mackey PE, Lyon PE, Rizzi TE, Ritchey JW. Complex mammary carcinoma with metastases to lymph nodes, subcutaneous tissue, and multiple joints in a dog. Vet Clin Pathol. 2018; 47: 477-483.

71. Lutful-Kabir, F, Alvarez, C, Bird R. Canine mammary carcinomas: A comparative analysis of altered gene expression. Vet Sci. 2015; 3: E1.

72. Yoshikawa Y, Morimatsu M, Ochiai K, Ishiguro-Oonuma T, Wada S, Orino K, et al. Reduced canine BRCA2 expression levels in mammary gland tumors. BMC Vet Res. 2015; 11: 159.

73. Muto T, Wakui S, Takahashi H, Maekawa S, Masaoka T, Ushigome S, Furusato M. P53 gene mutations occurring in spontaneous benign and malignant mammary tumors of the dog. Vet Pathol. 2000; 37: 248-253.

74. Queiroga FL, Pires I, Lobo L, Lopes CS. The role of Cox-2 expression in the prognosis of dogs with malignant mammary tumours. Res Vet Sci. 2010; 88: 441-445.

75. Queiroga FL, Alves A, Pires I, Lopes C. Expression of Cox-1 and Cox-2 in canine mammary tumours. J Comp Pathol. 2007; 136: 177-185.

76. Klopfleisch R, von Euler H, Sarli G, Pinho SS, Gärtner F, Gruber AD. Molecular carcinogenesis of canine mammary tumors: News from an old disease. Vet Pathol. 2011; 48: 96-116.

77. Borge KS, Nord S, Van Loo P, Lingjærde OC, Gunnes G, Alnæs GIG, et al. Canine mammary tumours are affected by frequent copy number aberrations, including amplification of MYC and loss of PTEN. PLoS One. 2015; 10: e0126371.

78. Yoshikawa Y, Morimatsu M, Ochiai K, Suzuki Y, Wada S, Taoda T, et al. Effects of the missense mutations in canine BRCA2 on BRC repeat 3 Functions and comparative analyses between canine and human BRC repeat 3. PLoS One. 2012; 7: e45833.

79. Bhimrao VA. Mutational Analysis of BRCA2 Gene in Canine Mammary Tumor. Mumbai, India. Bombay Veterinary College; 2017, 89 pp.

80. Sundararajan S, Ahmed A, Goodman O. The relevance of BRCA genetics to prostate cancer pathogenesis and treatment. Clin Adv Hematol Oncol. 2011; 9: 748-755.

81. Lee C, Kweon O. Mutations of p53 tumor suppressor gene in spontaneous canine mammary tumors. J Vet Sci. 2002; 3: 321-325.

82. Rivera P, von Euler H. Molecular biological aspects on canine and human mammary tumors. Vet Pathol. 2011; 48: 132-146.

83. Abdelmegeed SM, Mohammed S. Canine mammary tumors as a model for human disease (Review). Oncol Lett. 2018; 15: 8195-8205.

84. Nguyen F, Peña L, Ibisch C, Loussouarn D, Gama A, Rieder N, et al. Canine invasive mammary carcinomas as models of human breast cancer. Part 1: Natural history and prognostic factors. Breast Cancer Res Treat. 2018; 167: 635-648.

85. Carvalho MI, Pires I, Prada J, Lobo L, Queiroga FL. Ki-67 and PCNA expression in canine mammary tumors and adjacent nonneoplastic mammary glands: Prognostic impact by a multivariate survival analysis. Vet Pathol. 2016; 53: 1138-1146.

86. Choi HJ, Jang S, Ryu JE, Lee HJ, Lee HB, Ahn WS, et al. Significance of EZH2 expression in canine mammary tumors. BMC Vet Res. 2016; 12: 164.

87. Modiano JF, Ritt MG, Wojcieszyn J. The molecular basis of canine melanoma: Pathogenesis and trends in diagnosis and therapy. J Vet Intern Med. 1999; 13: 163-174.

88. Enjoji S, Yabe R, Fujiwara N, Tsuji S, Vitek MP, Mizuno T, et al. The therapeutic effects of SET/I2PP2A inhibitors on canine melanoma. J Vet Med Sci. 2015; 77: 1451–1456.

89. Gillard M, Cadieu E, De Brito C, Abadie J, Vergier B, Devauchelle P, et al. Naturally occurring melanomas in dogs as models for non-UV pathways of human melanomas. Pigment Cell Melanoma Res. 2014; 27: 90-102.

90. Neoplastic and non-neoplastic tumors. In: Miller WH, Griffin I, Campbell KL, editors. Muller and Kirk's Small Animal Dermatology, 7th ed. St. Louis, Mo.: Elsevier–Saunders, 2012. Chapter 20, pp. 774-843.

91. Teixeira TF, da Silva TC, Cogliati B, Nagamine MK, Dagli MLZ. Retrospective study of melanocytic neoplasms in dogs and cats. Brazil J Vet Pathol. 2010; 3: 100-104.

92. Mochizuki H, Kennedy K, Shapiro SG, Breen MB. BRAF mutations in canine cancers. PLoS One. 2015; 10: e0129534.

93. Lee BH, Neela PH, Kent MS, Zehnder AM. IQGAP1 is an oncogenic target in canine melanoma. PLoS One. 2017; 12: e0176370.

94. Lloyd KCK, Khanna C, Hendricks W, Trent J, Kotlikoff M. Precision medicine: An opportunity for a paradigm shift in veterinary medicine. J Am Vet Med Assoc. 2016; 248: 45-48.

95. Hendricks W, Zismann V, Sivaprakasam K, Legendre C, Poorman K, Tembe W, et al. Somatic inactivating PTPRJ mutations and dysregulated pathways identified in canine melanoma by integrated comparative genomic analysis. PLoS Genet. 2018; 14: e1007589.