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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.

Keywords
Dogs; canine; cancer; lymphoma; mast cell; mammary gland; melanoma; osteosarcoma

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
	ENSCAFG00000031638	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

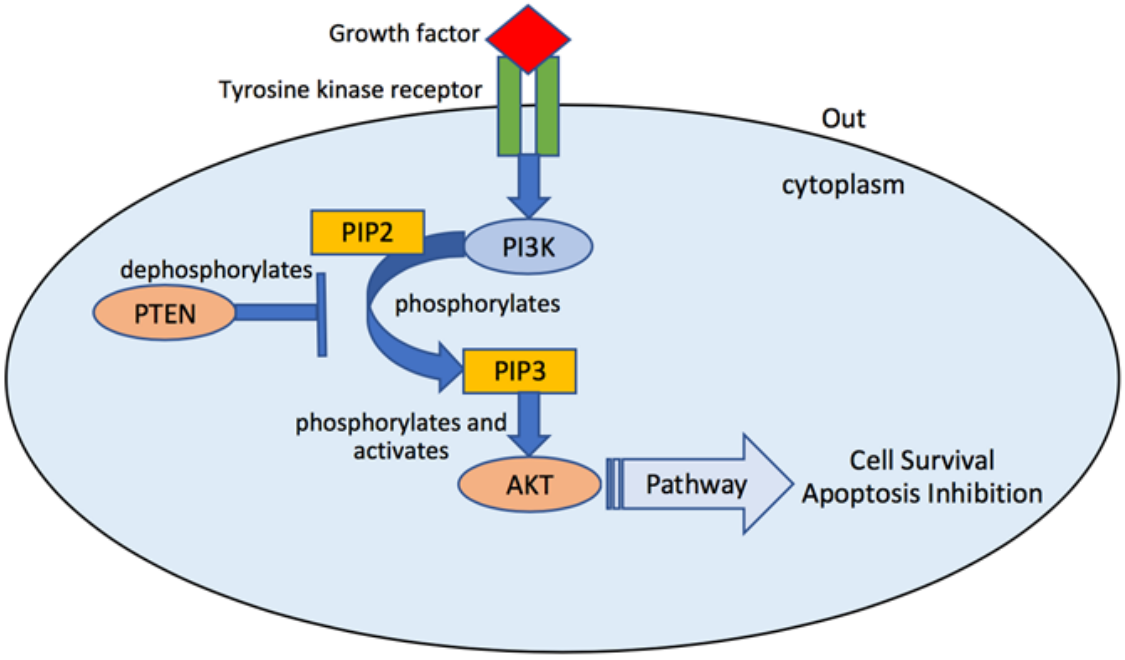

Figure 1 PI3K–AKT–mTOR pathway. When a growth factor ligand binds to a receptor tyrosine kinase, it activates PI3K which phosphorylates PIP2 to become PIP3, which then activates AKT to cause cell survival and decreased apoptosis. PTEN antagonizes this pathway by dephosphorylating PIP3. Thus, mutations 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].

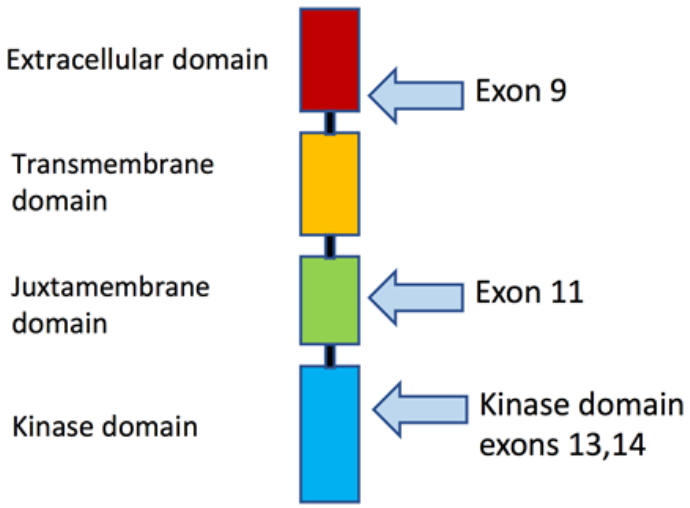

Figure 2 Mutations were identified in exon 11 of *c-KIT*, which codes for the juxtamembrane portion of KIT. Reproduced from the open access source [48].

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].

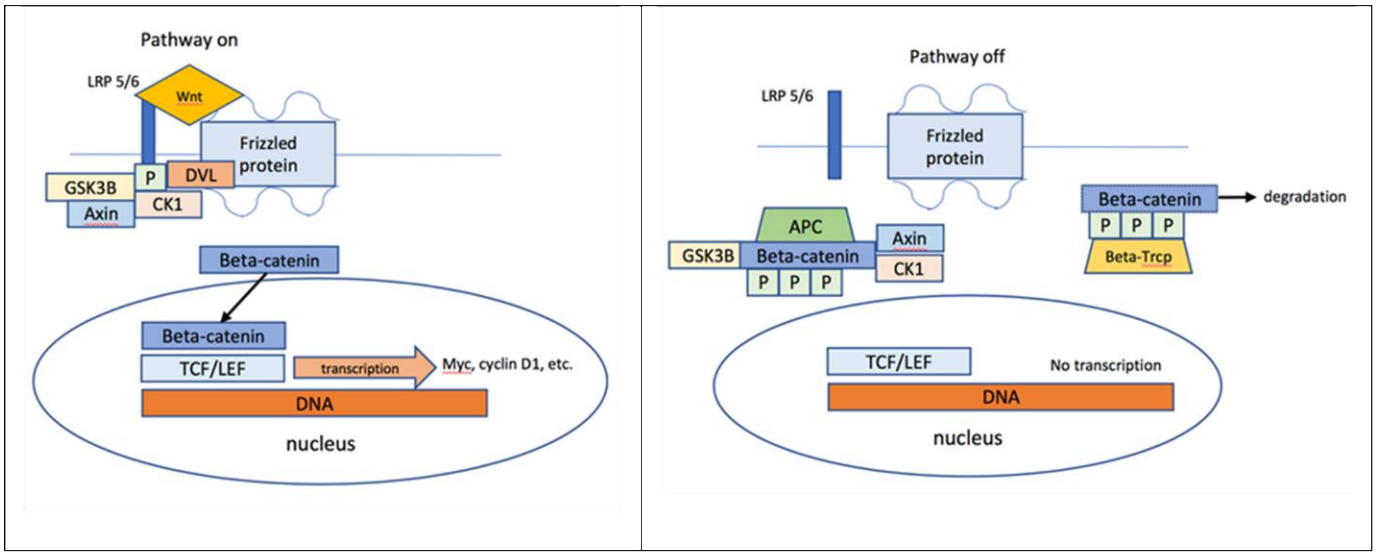

Figure 3 Wnt signaling pathway. When Wnt binds to a frizzled protein, the Wnt pathway is activated and beta-catenin is able to facilitate the transcription of key genes such as proto-oncogene myc. When the Wnt pathway is off, beta-catenin is phosphorylated and tagged for destruction by beta Trcp. Reproduced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

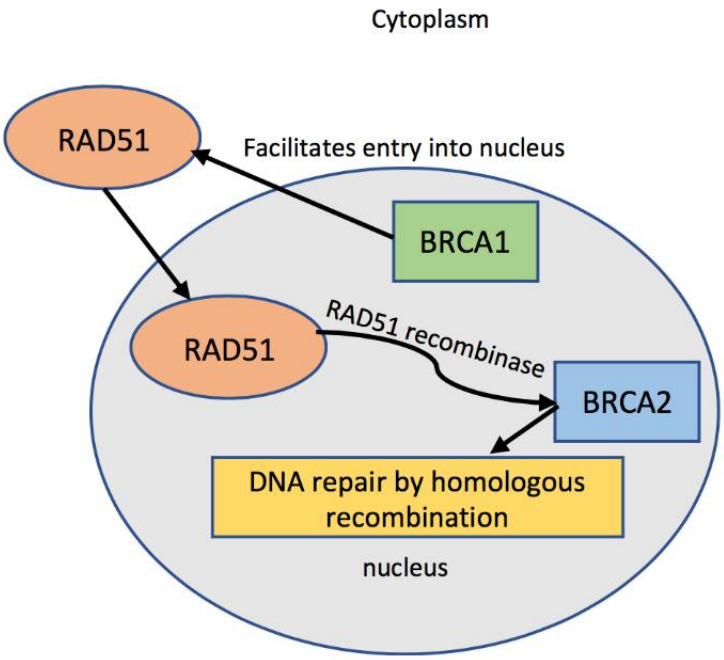

Figure 4 Interaction between BRCA2 and Rad51 in homologous recombination DNA repair (all related to reproduction) Reproduced from the open access source [80].

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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