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Genetics of canine cancer: a guide for the veterinary oncologist

Maja Louise Arendt[1,2,3*], Anna Darlene van der Heiden[2,3], Raphaela Pensch[2,3] and Kerstin Lindblad-Toh[2,3,4]

Abstract

Cancer is driven by acquired genetic aberrations that drive the cellular cancer phenotype. In addition, hereditary genetic risk factors play a central role explaining the large difference in cancer risk between different dog breeds. There has been a revolution within genetic research over the past decades facilitated by technological advances and reduced costs. We can use sequencing technologies to characterize genetic changes in cancer cells and identify markers to diagnose and differentiate cancers. In addition, these technologies can lead to the identification of druggable targets, leading to advancements within cancer therapy. This review describes some of the advances within oncogenetics in companion dogs and provides an overview of published genome wide association studies investigating predisposing genetic risk factors as well as studies investigating somatic cancer-driving mutations in dogs.

Introduction

For morethan a century it has been known that genetic aberrations play a role in cancer [1]. However, it is only during the past 20 years that research tools have become readily available to characterize the genetic changes inside cancer cells at a base-pair level at high scale. The field of cancer genetics has been fostered by rapid advancements in DNA sequencing technology and analytical tools parallelized by a logarithmic reduction in cost for these technologies [2]. Within human oncology, genetic test modalities are now widely applied with the view that this can improve patient management. Examples of genetic testing, that are applied in the clinical setting, are testing for germline predisposing variants in families with increased cancer burden, classification of cancer subtypes based on specific mutations and therapeutic choice based on cancer mutation profiles [3–5]. The World Health Organization classification guidelines now include an array of genetic aberrations which are used to distinguish different subtypes, guide management and predict prognosis in human patients [6, 7]. Within veterinary oncology there has been a strong drive to sequence cancer types in dogs which serve as good comparative models for human cancer research [8–10]. Similarly, there has been a drive for identifying genetic risk factors with the view that these findings even can inform human research and increase our understanding of cancer biology [11–13]. With cancer being the leading disease related cause of death in dogs, technologies which can improve detection, diagnosis, therapy and monitoring are warranted [14]. Up until recently, the application of genetic testing in the veterinary oncology clinic has been limited. We are now entering an era where the advancement of sequencing methods at reduced cost allows for application of these test modalities in the clinical veterinary setting. This paper provides an overview of some of the genetic discoveries and tools that are being applied to companion animal oncology. It focusses on the identification of genetic risk factors predisposing to

*Correspondence:
Maja Louise Arendt
maja.arendt@sund.ku.dk
[1] Department of Veterinary Clinical Sciences, University of Copenhagen, Copenhagen, Denmark
[2] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
[3] SciLifeLab, Uppsala University, Uppsala, Sweden
[4] Broad Institute of MIT and Harvard, Cambridge, MA, USA

cancer in dogs and detection of somatic mutations driving cancer. Finally, we provide insights into how genetic tools can be used to support diagnostics or decision making in a clinical setting in the future.

The canine genome

A foundation for performing genetic research is a high-quality annotated reference genome. Since the first canine reference genome was released in 2004 (canFam1), there have been multiple efforts to enhance the genome by filling gaps and improving the annotation resulting in the canFam2 and canFam 3.1 [15, 16]. Annotation is central to the use of a reference genome, as it determines which regions of the genome encode genes and other important functional regulatory elements. The first canine reference genome was based on paired-end shotgun sequencing of multiple library sizes including bacterial artificial chromosome libraries (BAC) [15]. Recently, with the development of first short read data such as Illumina and then long read sequencing technologies such as PacBio and Chromium 10X, it has become possible to generate new and improved reference genomes with fewer gaps and more contiguous sequences covering complex and important genome regions, such as the dog leukocyte antigen (DLA) loci [17, 18].

The original reference genome was built on the genetic sequence from a female boxer, whereas some of the recent canine reference genomes have been based on other dog breeds, such as German shepherd (UU_Cfam_GSD_1.0/canFam4), great Dane (UMICH_Zoey_3.1/canFam5) and Labrador retriever (ROS_Cfam_1.0) [15, 17, 19, 20]. It has been suggested to use an outgroup such as the wolf as a reference to reduce the risk of bias related to variant calling when comparing to a single breed [21]. For research into germline genetic variants and how these can predispose to a disease, the reference genome is of particular importance. Boxers have an increased risk for certain neoplastic disorders such as lymphoma, mast cell tumors and glioma, hence there is a risk that the founder of this reference genome could be representing risk factors [10, 22, 23]. In this context Tasha, the boxer donating material for the original reference genome, died of cancer. When aligning sequencing data to a reference genome only variants differing from the reference genome are usually evaluated. This means that genetic risk variants could be overlooked if these are represented by the reference genome. Although all the genome builds are of high quality, from an oncological perspective, the UU_Cfam_GSD_1.0/canFam4 was specifically generated with the view to promote comparative cancer research between dogs and humans, and 282 Tier1 and 78 Tier2 cancer census genes were completed in this genome [17, 24]. As each reference genome reflects the genetic diversity within a specific individual, it does not adequately represent the genetic diversity across multiple individuals or different dog breeds. In other species, this problem has been solved by creating pangenomes. These reference genomes represent the diversity across a population and provide an improved representation of genetic variation and haplotypes within a species [25–28]. Although a pangenome has not yet become available for dogs, it is anticipated that it will be in the near future. The application of a canine pangenome to genetic research will likely improve our understanding of genetic diversity within dogs and our ability to detect structural genetic variants. When evaluating genetic variation, both in relation to germline variation and somatic tumor mutations, it is important to distinguish between genetic variants with a functional role, often referred to as driver mutations, versus genetic variants without a functional role, also known as passenger mutations. Distinguishing between driver and passenger mutations requires in depth understanding of the functional implications of the variant and its putative role in disease pathogenesis.

Genetic studies in dogs are often performed with a comparative intent, as therapeutic targets can be shared across species, and knowledge learned from one species can be valuable for the other species, hence promoting the cancer field overall. In this sense, it is important to acknowledge that, although there is a high level of homology between the human and the canine genome, there are also clear interspecies differences, such as genes inactivated in one species but not the other, or gene families that differ in their sequences and number of active genes [29]. The *PRDM9* gene which has been shown to have a potential role in human cancer is an example of a gene which has been shown to be inactivated in the dog genome [30]. The Cytochrome P450 gene families implicated in drug metabolism are likewise a group of genes where species differences are known and where functional differences potentially can implicate the translation of therapeutic trials [31]. However, most of the genes important for cancer predisposition or that are commonly mutated within cancer cells are highly conserved across species with high interspecies homology [29]. The canine genetic makeup compared to the human counterpart is summarized in Tables 1 and 2.

Inherited genetic risk factors

Differences in cancer frequency between different dog breeds have provided evidence that heritable genetic risk factors likely play an important role in the development of cancer in dogs [35]. This becomes particularly noticeable when looking at specific cancer types such as urothelial cell carcinoma which is observed more frequently in Scottish terriers and histiocytic sarcoma

Table 1 Comparison of the genome size, chromosomal arrangements and number of protein coding genes between species

Genome comparison	Canine genome	Human
Number of chromosomes	39	23
Genome size	2.5 billion bp	3.1 billion bp
Protein coding genes	21,063[a]	19,868[a]

[a] The number of protein coding genes is based on the Ensembl (version113) annotation summary for the canine genome assembly UU_Cfam_GSD_1.0/canFam4 and the human genome assembly GRCh38.p14 [32]. Differences in number of protein coding genes between species can in part be explained by more detailed annotation of the human assignment of larger repertoire of non-coding genes [33].

Table 2 Overlap in the genomic sequence between the human (GRCh38) and canine (canFam4) reference genomes from the 241 mammals alignment

Genomic sequence overlap	Whole genome	Coding DNA sequence(CDS)
Percentage of the human genome (reference) covered by the canine genome	84.0%	90.1%
Percentage of the canine genome (reference) covered by the human genome	81.7%	91.8%

Numbers have been generated by Michael Dong, Uppsala University [34]

which is observed more frequently in flat-coated retrievers, with odds ratios of 18.1 and 62.0, respectively, compared to other dog breeds [36, 37]. The exact mode of inheritance for cancer within individual dog breeds has not been determined. Complex inheritance caused by one or multiple low penetrance risk alleles has been proposed as well as simpler Mendelian inheritance caused by risk alleles with high penetrance [38–40]. To identify genetic risk factors, genome wide association studies (GWASs) using either broad single nucleotide polymorphism (SNP) panels or imputed low-pass whole genome sequencing (WGS), are being performed. In these studies, the genotypes of comprehensively phenotyped cases and controls for a given disease are compared to identify genomic loci that harbor genetic variation associated with disease risk [41, 42]. GWASs using SNP panels have the advantage that the genotyping is usually robust. However, the disadvantage is that there is a risk that there are no SNPs in the selected panel that tag the genetic variation associated with the disease phenotype [43]. Another disadvantage is the identification of often very large associated regions, making it difficult to identify causal risk alleles. Low pass sequencing is a method where the whole genome is sequenced at a low coverage (usually 0.5 to 1×coverage). This coverage is a mean across the approximately 2.5 billion base pairs which make up the dog genome. This results in regions with no coverage and other regions with deeper coverage. As there are many positions in the genome which are heterozygotic, this low coverage means that for most positions only one allele is represented. Imputation refers to the statistical prediction of missing genotypes based on known haplotypes from a reference database built on deep sequencing of many diverse dogs. In general, to confidently evaluate the true genotypes across the genome a sequencing depth of ~ 30×coverage is applied. The advantage of low-pass sequencing is the evaluation of more genetic variants across the whole genome at a lower cost than deep sequencing. However, there is a risk that rare variants are missed if not represented adequately by the imputation panel and that there is a noteworthy discordance between the true genotype and the imputed genotype [44].

Most commonly, GWAS analysis has been performed within a particular dog breed, as it is uncertain whether genetic risk factors for the same disease are shared between breeds of different origin. Even dogs within the same breed but with different geographical origin can show differences in the importance of different risk loci. Two studies, including dogs from Europe and North America, investigating predisposition to mast cell tumors and histiocytic sarcoma in golden retrievers and Bernese mountain dogs respectively, found that the dominating risk locus was different between the North American and European dogs, when analyzed separately [45, 46]. The majority of canine cancer GWASs have focused on analyzing breeds separately (Table 3). However, multibreed GWASs have been performed for some traits. These require balancing of cases and controls across breeds and taking the multibreed origin into account in the analysis, to avoid identifying loci reflecting breed rather than disease, and to avoid overcorrection of true associations not present across all included breeds [47]. There are mathematical models that can be applied to account for population structure and the effect of multiple risk alleles in multibreed studies [23, 48].

Results from several published GWASs have identified risk loci associated with different types of cancer in specific or across several dog breeds (Table 3). Although many studies have identified significant genetic risk loci related to the risk of developing a specific type of cancer, only a few studies have identified putative functional variants explaining the disease risk. Interestingly, overlapping risk loci on chromosome 5, 11, 14 and 20 have been identified in independent studies with different dog breeds, dog populations and even different cancer types, emphasizing that predisposing risk loci can be shared across multiple breeds and could predispose to multiple different cancer types [12, 13, 45, 50, 52, 54, 55, 58]. With

Table 3 Summary of selected published genome wide association studies in different canine tumors

Author /Year	Breed (s)	Cancer	Lead SNP from GWAS	Genes highlighted as putatively implicated in disease risk
Arendt 2015 [45]	Golden retriever	Mast cell tumors	US Chr14:11,765,081 EU Chr20:45,596,213 Combined Chr14: 11,721,433, 11,807,161 (equally associated)	Chr14: *SPAM1, HYAL4, HYALP1* Chr20: *HYAL1, HYAL2, HYAL3, GNAI2*
Biasoli 2019 [49]	Labrador retriever	Mast cell tumors	Chr31:34,694,234	Chr31: *DSCAM*
Evans 2021 [50]	Flat-coated retriever	Histiocytic sarcoma	Chr5:33,001,550 Chr19:(conditional, heterozygotes for Chr5 lead SNP):52,487,724	Chr5: *PIK3R6* Chr19: *TNFAIP6*
Hayward 2016 [51]	Labrador retriever	Mast cell tumors	Chr36:16,889,272	Chr36: *ITGA6*
Hedan 2021 [52]	Bernese mountain dog (BMD) Golden retriever (GR) Flat coated retriever (FCR) Rottweiler (RW)	Histiocytic sarcoma (HS) Mast cell tumor (MCT) Lymphoma (LSA)	BMD HS Chr11:41,161,441 BMD HS LSA Chr11:41,161,441 BMD GR LSA HS Chr5:32824053[a] BMD HS MCT Chr11:41,161,441 BMD GR HS MCT Chr20:33321282[a] BMD HS Chr11:41215628[b] BMD FCR HS Chr11:41252822[b] BMD FCR RW Chr11:41252822[b]	Chr1: *RSPH3*(other locus than lead SNP) Chr2: *PFKFB3* (other locus than lead SNP) Chr5: *SPNS3, TRPC6, BORCS6*, Chr11: *CDKN2A/B, CDKN2B_AS1, MTAP, CAAP1, TUSC1, C9orf72* Chr14: *POT1, PSMD4, LEP, CPA1,* Chr20: *FHIT, ARHGEF3, IL17RD, C3orf67* ChrX: *TBL1X, SHROOM*
Karlsson 2013 [13]	Greyhound (GH) Rottweiler (RW) Irish wolfhound (IW)	Osteosarcoma	GH Chr11:41,375,800 RW Chr1:113,616,670 IW Chr5:12,259,066	Chr11: *CDKN2A/B* (Shared locus across breeds) Chr1*: Multiple genes in this locus* Chr5*: BLID*
Karyadi 2013 [53]	Standard poodle	Digital squamous cell carcinoma	Chr15:29,371,013	Chr 15: *KITLG*
Labadie 2020 [54]	Golden retriever	T-Zone Lymphoma (TZL) Mast cell tumors (MCT)	TZL Chr8:53,818,371 (TZL) TZL + MCT Chr14:11794735[a]	Chr8: *DIO2, TSHR* Chr14: *SPAM1, HYAL4, HYALP1*
Letko 2021 [55]	Leonberger	Osteosarcoma	Chr11:39,434,964	Chr11: CDKN2A/B
Melin 2016 [11]	English springer spaniel	Mammary tumors	Chr11:73,290,522	Chr11: *CDK5RAP2*
Mortlock 2023 [56]	Bullmastiff	Lymphoma	Chr33:8,104,361	Chr33: *SENP7, NFKBIZ,* Chr13: *MYC* (other locus than lead SNP)
Parker 2024 [48]	Shetland Sheepdog (SS) Multiple breeds	Urothelial cell carcinoma	SS alone and SS + multiple breeds Chr13:44,493,602, 44,508,476, 44,520,164 (SNPs equally associated)	Chr13: *NIPAL1*
Shearin 2012 [46]	Bernese mountain dog	Histiocytic sarcoma	EU Chr14:11,076,261 US Chr11 38,330,565 Combined Chr11:38,330,565	Chr11: *CDKN2A/B, MTAP*
Soh 2023 [57]	Border collie	Lymphoma	Chr18: 18:38,704,682	Chr18: *DLA79,* Chr27: *WNT10B, LMBR1L, KMT2D, CCNT1* (other locus than lead SNP)
Tonomura 2015 [12]	Golden retriever	Hemangiosarcoma (HSA) B cell lymphoma (BLSA)	HSA Chr5:29,892,306 BLSA Chr5:33,845,636 HSA + BLSA Chr5:29,892,306	Chr5: *TRPC6, STX8*
Truve 2016 [23]	Multiple breeds	Glioma	Chr26:9,780,187	Chr26: *CAMKK2, P2RX7, DENR*
Zapata 2019 [40]	Greyhound (GH) Rottweiler (RW) Irish wolfhound (IW)	Osteosarcoma	RW IW Chr1:1,136,161,670 GH RW IW Chr25:16,672,073	Chr25: FGF9 shared locus across breeds[a] Genes nominated in other loci: *CDKN2A/B, AQP4, OTX2, EWSR1 retrogene, BMPER, MTMR7, MARCO, NELL1, FBRSL1, IGF1, MTMR9, TANGO2*

The table summarizes the author information, breeds involved in the study, cancers / tumor types studied and location of the most associated SNP, as well as putative genes suggested to be implicated in the disease. Positions are shown on CanFam3.1

[a] Indicates that data recycled from other publications have been used in this analysis

[b] Analysis performed with a larger imputed SNP dataset

improved knowledge of the genetic variation across dogs and the establishment of large scale canine reference databases such as the Dog10K, it is anticipated that the identification of functional risk alleles will be less challenging in the future [20].

Acquired mutations in cancer cells

Cancer is driven by somatic mutations leading to an altered cellular phenotype with dysregulated signaling pathways in a clonal population of cells. Whilst some cancers are mainly driven by small genetic alterations in key tumor suppressor and oncogenes, other types of cancer are characterized by larger genomic alterations such a chromosomal duplications and structural alterations [59–61]. The rapid drop in price for sequencing has made it possible to characterize the genetic changes that occur in cancer cells in humans as well as dogs and other species [62–64]. Initially, most studies focused on sequencing the exonic (protein coding) part of the genome, known as whole exome sequencing (WES). As the coding part of the genome accounts for less than 2% of the genome, this massively reduces the sequencing cost and the amount of data needing to be analyzed [65, 66]. Different canine exome panels have been used to capture exons and, in some instances, also part of the up and downstream regulatory sequences, across the whole genome [8, 10]. As the knowledge on cancer-driving genes and mutations has advanced, smaller exome panels have been introduced capturing selected panels of genes that have been shown to be recurrently mutated in cancer cells [67, 68]. One such example is the commercially available Canine Search light exome panel, which selectively captures 120 genes known to be highly relevant in cancer [69]. This panel can be run as a clinical test on patient material and it has been shown that it is possible to run this on both formalin-fixed paraffin-embedded material, needle aspirates as well as frozen biopsies, facilitating practical clinical application [69]. A comprehensive overview of genes that were identified to be recurrently mutated in canine cancers by different sequencing methodologies is provided in Table 4.

There are many similarities between the mutation profiles of human and canine cancer cells. This is reflected by an overlap in significantly mutated genes within specific cancer types, i.e., genes affected by mutations more than would be expected by chance [81]. As an example, *TP53* is significantly mutated in both humans and dogs with osteosarcoma, indicating that alterations in this gene play an important role in driving osteosarcoma development [9, 72]. In addition, there is also an overlap in hotspot mutations between species characterized by specific mutations affecting conserved DNA base pairs leading to the same protein alteration. One example of a hotspot mutation is the PIK3CA p.H1047R, which is observed in both dogs and humans with mammary neoplasia [8]. The overlapping mutational spectra between species reflect shared cancer biology and evolutionarily conserved cellular selection pressure, i.e., for a cancer to develop relatively distinct genetic events need to occur that offer a selective advantage for the particular cancer type. This overlap also facilitates comparative clinical trials in the era of precision medicine. Although there are clear similarities between species, we also observe species differences. As an example, the tumor suppressor gene *SETD2* has been shown to be significantly mutated in canine multicentric lymphoma and osteosarcoma, although it is not known as a major cancer driver within the human orthologous diseases [9, 73].

Recently, researchers have started using WGS of tumor / normal pairs, as the price difference between sequencing of exome capture libraries and WGS libraries has decreased. In addition, parallelized computation models reduce the analysis time. WGS improves the detection of genomic rearrangements and structural variants as there are no gaps in the sequencing coverage of the genome [82]. Only approximately 1–2% of somatic mutations cause changes in the protein coding sequence, whilst the remaining mutations are located within the non-coding part of the genome [83]. Less attention has been paid to non-coding mutations in the past. This is partly because of difficulties assigning non-coding mutations with a functional role. However, one might assume that ~ 10% of the genome has a regulatory function determining when and where proteins should be made [84]. Hence, distinguishing functional cancer driving mutations from passenger mutations is key [85]. Non-coding mutations can play a role in regulating gene expression by affecting enhancer or promoter regions, splice signals and topologically associated domains (TADs) without changing the protein coding sequence [85]. Based on that, one can expect that genes for which the cancer cell relies on overexpression, without alteration of the protein coding sequence, will be subject to non-coding regulatory mutations. For example, there are frequent non-coding mutations upstream of the *TERT* gene in human cancer cells, leading to overexpression of telomerase without alterations in the coding sequence [86]. Multiple tools have been developed to characterize the non-coding part of the genome and assign mutations with a candidate role [85]. One approach is using evolutionary constraint scores. In the Zoonomia project, scores were developed by comparing DNA sequences across more than 200 mammalian species to assign putative functional roles to single base pair positions across the whole genome [29, 34]. This approach makes it possible to distinguish mutations that are likely to have a functional impact in

Table 4 Selected publications characterizing somatic mutations in canine tumors

Author	Cancer / Tumor type (number of tissues)	Method	Recurrently mutated genes
Amin 2021 [70]	Glioma (83)	WES/WGS	*PDGFRA, PIK3CA, NF1*
Arendt 2023 [8]	Mammary tumors (55)	WES	*PIK3CA, MUC1, KRAS, TTN, NLRP5, ENSCAFG00000038503, ARID1A*
Das 2023 [71]	Soft tissue sarcomas (29)	WES	*TP53, KMT2D*
Elvers 2015 [10]	B and T- cell lymphoma (105)	WES	***T-cell**: PTEN, SATB1, MAP2K1, PSMA1, COX8A, LTA4H, TBC1D26, PTPN6, NLRP5, GLUD2, KRTAP10-6, ENSCAF00000031638* ***B-cell**: FAM90A1, DDX3X, TRAF3, PSMA1, POT1, FBXW7, TP53, PNRC1, TBC1D26, RPL23A, SETD2, ENSCAFG00000031638*
Gardner 2019 [72]	Osteosarcoma (37)	WES/WGS	*TP53, SETD2, RPL27A, MLLT10*
Gianuzzi 2022 [73]	Diffuse large B-cell lymphoma (77)	WES	*TRAF3, SETD2, POT1, TP53, MYC, FBXW7, DDX3X, TBL1XR1, MAP3K14, ENSCAFG00000046771, PHC3, ABCA13, CIC, LRP1B, TTN, RARA, PIK3CD, H3C8, EHD3, GBE1, VWF, DIAPH2, FAM50A, GADD45A, SYNE1, THBS2, PLEC, ETV1, HIVEP3, MYT1L, LRRN3, MEF2C, ATXN1, KIF21A, TLR5, FSIP2, KDM6A, TRRAP, SYNE2, SUZ12, LAMA1, ANKRD11, LRRIQ1*
Kim 2020 [74]	Mammary tumors (143 malignant + 40 benign)	WES	*PIK3CA, KRAS, MKI67, TP53, NKX1-2, SETD1A, PTEN, PIK3R1, AKT1*
Lee 2019 [75]	Mammary tumors (20)	WES	*PIK3CA*[a], *PRMT3, cOR8S14, ENSCAFG00000020185, ENSCAFG00000029433, BHLHA9*
Lorch 2019 [76]	Pulmonary carcinoma (5) Additional 73 tumors and 10 cell lines captured by small selected array)	WES (5 dogs)	*HER2, TP53, PTEN*[a]
Megquier 2019 [77]	Hemangiosarcoma (47)	WES	*TP53, PIK3CA*[a], *PIK3R1, ORC1, RASA1, ARPC1A*
Sakthikumar 2019 [9]	Osteosarcoma (66)	WES	*TP53, SETD2, TANGO2, LOXHD1, MYT1L*
Saffari 2019 [78]	Ameloblastoma (16)	WES	*HRAS*
Thomas 2023 [79]	Urothelial cell carcinoma (8 BRAF p.V595E + , 28 BRAF p.V595E -)	WES	*BRAF, MAP2K1, LRP1B, SMCHD1, ARID1A, CSMD3, KDM5C, CSMD1, RYR2, KMT2D, STAG2, MSH6, PBRM1, ATM, MDC1,*
Wang 2017 [68]	Hemangiosarcoma (20)	WES	*TP53, PIK3CA*
Wong 2019 [62]	Oral malignant melanoma (65)	WES	*NRAS, TP53, RP1, FAT4, PTPRJ, CSMD3*
Wong 2023 [80]	Urothelial cell carcinoma (87)	WES	*BRAF, TTN, ZFHX4, CSMD3, FSIP2, CDH12, USH2A, LRP1B, HMCN1, ARID1A, PCDH17, HMCN2, TNNI3K, MGAM2, LRP2, ENSCAFG00000007873, KDM6A, LAMA2, FLNA, MDN1, GRIK2, COL11A1, VCAN, XIRP2, ZNF536, ZNF804B, TENM3, DNAH7, PCDH9*

The table summarizes the author, cancer or tumor types implicated in the study, target of sequencing (whole exome sequencing (WES), whole genome sequencing (WGS)) and genes found to be mutated in >5% of tumors and in at least three individuals. Differences in data curation and filtering as well as methods for determination of significantly mutated genes are variable across studies

[a] Data based on sequence capture of selected genes

cancer. Using this approach, it has been possible to identify genes enriched for non-coding constraint mutations in tumor / normal sequencing data from human medulloblastoma and glioblastoma [87, 88]. This methodology is currently being applied to ongoing canine and human cancer sequencing studies.

Aside from understanding which genetic changes drive cancer, sequencing of cancer tissue can also be used to understand what causes the mutational process by evaluating the mutational signature. Combinations of mutation types reflect underlying extrinsic or intrinsic causes. Those can be infidelity of DNA replication and the accumulation of mutations during aging as well as heritable genetic defects in DNA repair or exposure to environmental carcinogens such as UV light, tobacco smoke or ionizing radiation [89]. In dogs, which have a shorter lifespan and shorter time of exposure to carcinogens, it has been shown that the aging signature is the most dominant signature across different cancer types [9, 63]. There is, however, evidence that some tumors carry a signature that, in humans, has been related to UV light exposure [63]. In addition, a mutational signature that has not been characterized in humans and which appears to be frequent in cancers from golden retrievers, has also been identified [9, 63]. One could speculate that this signature is caused by germline genetic variants segregating within this breed that are predisposing to the mutational process, though this link has not been confirmed.

Clinical applications of DNA technology in veterinary oncology

Although we are still in the early phase of characterizing genetic variants that predispose to cancer, as well as identifying somatic cancer-driving mutations in companion animals in depth, sequencing and genotyping technologies offer enormous prospects for clinical use (Fig. 1).

Germline screening of risk variants

Identifying germline variation predisposing to cancer is an important tool to understand underlying causes of cancer. This can be used for selective breeding away from risk carriers, to identify individuals at risk of developing early onset disease who could benefit from entering a screening program and to identify potential markers for preventing disease or reducing risk. However, with few exceptions, we are not yet in a position where we prospectively and reliably can predict cancer risk in dogs based on genetics [90]. The canFam 3.1 chr5:42,186,445 A > G autosomal dominant genetic variant causing renal cystadenoma and nodular dermatofibrosis in German shepherds is an example of a genetic variant that can identify which dogs carry risk factors for this neoplastic disorder [90]. A genetic test for cancer associated risk alleles is available to guide the selection of Bernese mountain dogs before breeding [91, 92]. Though this test has been available for several years, we are still awaiting data showing the long term effects of this test. Before applying a genetic test to a clinical setting retrospective validation in independent patient cohorts and functional validation of genetic cancer predisposing variants should be performed. As for now, breed appears to be the most reliable risk predictor [35]. However, with larger initiatives and validation studies, improved tests for risk prediction could become available, which preferable can be used within and across breeds. In addition to determining individuals at risk of disease, germline genetic profiling can also be used to predict individuals at risk of developing severe adverse effects or having an altered response to antineoplastic drugs. A well characterized four base pair deletion in the *ABCB1* gene has been shown to increase the risk of severe chemotherapy

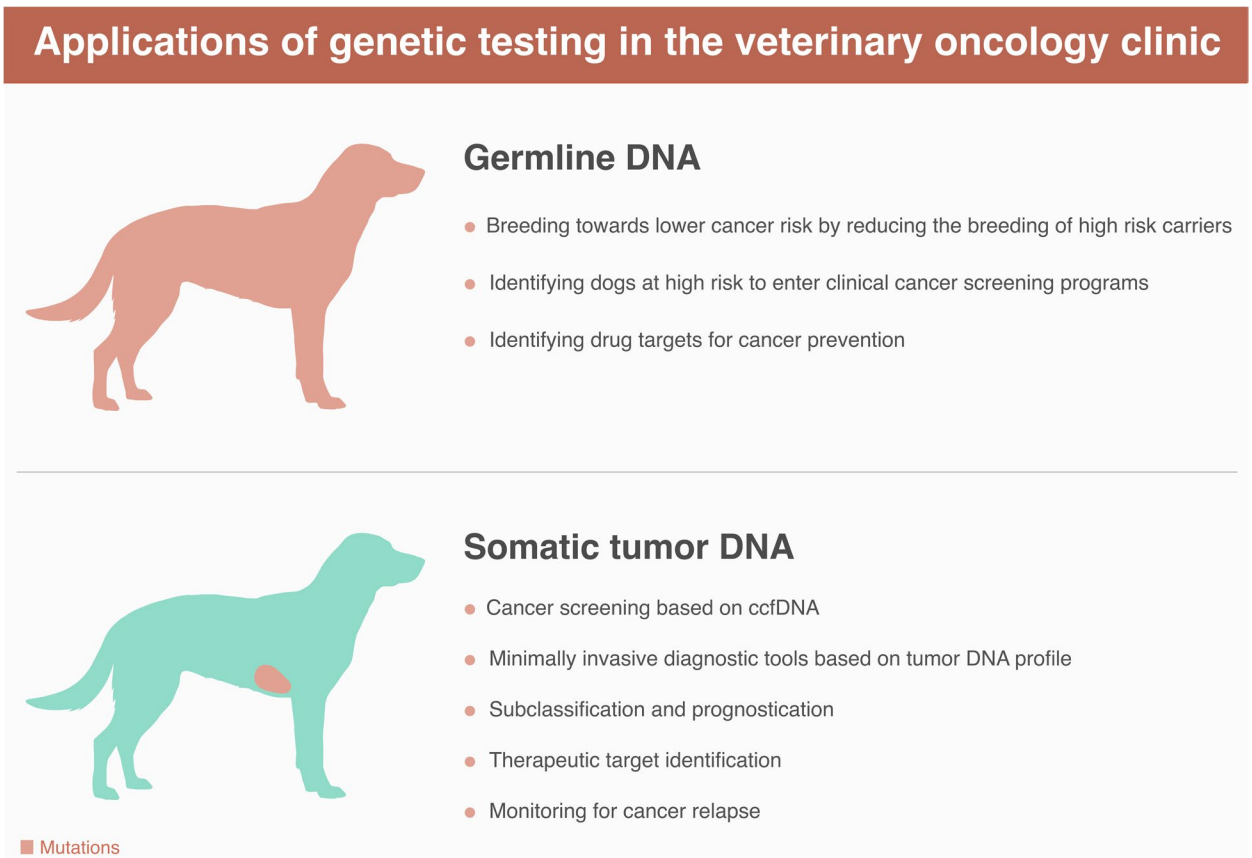

Fig. 1 Some of the applications of genetic testing in the veterinary oncology clinic. Genetic testing holds great promise in the oncology clinic as a tool for identifying individuals at greater risk of developing cancer as well as characterizing cancers once they have developed. Circulating cell-free DNA (ccfDNA)

induced neutropenia in dogs receiving drugs which are transported by the *ABCB1* encoded p-glycoprotein transporter [93, 94]. In humans a larger panel of genetic variants have been characterized which can influence the response or risk of adverse effect from chemotherapy and other therapeutic modalities which suggest that more could be discovered in dogs [95, 96].

Somatic mutations for screening, diagnosis and targeted therapy

The detection of somatic mutations in cancers has a solid clinical application and can be applied as a tool to screen, diagnose, classify and monitor neoplastic disease.

Early detection of circulating cell-free DNA (ccfDNA) from cancer cells, that is short DNA fragments around 160 nucleotides in length originating from cells undergoing apoptosis, offers the opportunity for cancer screening and early detection as well as providing a minimally invasive tool to aid in confirming a diagnosis of cancer [97].

Being able to characterize small amounts of cancer cell DNA from fine needle aspirates or fluid samples containing cancer cells provides the possibility to distinguish reactive processes from neoplastic processes and perhaps even assigning a provisional diagnosis, reducing the need for larger, more invasive biopsies [98]. The urine sediment-based *BRAF* mutation screening test for urothelial and prostatic carcinoma in dogs is already widely applied with a reported sensitivity of up to 85% and a specificity reaching 100% [99]. It aids in confirming a diagnosis without the need for larger tissue biopsies, which increase the risk for complications and potentially cancer seeding [99, 100]. This test is facilitated by the strong dependency of urothelial carcinoma on the activation of the *BRAF* oncogene and the conserved activation hotspot of this gene, allowing for detection by PCR based methods [99]. Further the test is enabled by the abundant exfoliation of cancer cells directly into the urine. By combining large-scale sequencing data from canine cancer with clinical records and patient outcomes, we might be able to identify prognostic predictors for patient outcome. In addition, major cancer-driving genes could also provide novel therapeutic drug targets and provide tests to select which patients are likely to respond to a given therapy. One such example already existing in veterinary oncology is the veterinary licensed drug masitinib (Masivet, AB Science, France). It was licensed in Europe for use in dogs with non-resectable mast cell tumors that carry activating mutations in the *KIT* oncogene, based on evidence from an initial clinical trial that showed significantly prolonged time to progression (TTP) in dogs with *KIT* mutated tumors [101]. In the study the median TTP for dogs with *KIT* mutated tumors was 241 days compared to 83 days for the placebo treated control group. In comparison the median TTP was only 141 days for dogs without *KIT* mutated tumors treated with masitinib [101].

A study investigating the prognostic value of the SearchLight DNA™ sequence capture panel, showed that certain genetic aberrations were associated with a worse outcome suggesting that the panel could offer prognostic information. In addition, the study also showed an improved outcome in dogs receiving treatments chosen on the basis of their tumor's mutation profile. Though this sounds promising, it should be taken into account that the study included 127 dog representing 26 cancer types and hence in such a heterogenous dataset there could be other parameters influencing outcome [67]. In humans, precision medicine, as in targeted treatment for specific mutation profiles in cancer cells, is being widely applied with a large panel of drugs approved for specific mutations [102]. Another clear role for applying genetic methods in the oncology clinic is the easing of staging procedures and minimally invasive screening for disease relapse. We already see several commercial applications of genotyping and sequencing technologies on the veterinary market for both cancer screening as well as cancer characteristics and drug target identification [67, 103, 104]. These tests hold great promise for improving the workflow in the oncology clinic and allowing companion animals to enter the era of precision medicine. We need to consider that the research data available for companion animals is still limited. Long-term validation studies are needed to validate the precision of these tests. It is also important to determine whether early cancer detection will lead to an overall survival benefit in veterinary cancer patients. Furthermore, caution should be taken in the interpretation of single point mutations. For instance, we know that there is an overlap between some of the significantly mutated genes and hotspot mutations between benign and malignant disease. One such example is the *PIK3CA* hotspot mutations which are frequent in both benign and malignant canine mammary tumors as well as hemangiosarcoma [8, 68]. More than a million human cancer samples have been characterized by mutational profiling leading to genetic testing now being widely applied in human oncology [24]. In comparison the number of cancer samples in dogs which have been characterized are sparse and the integration of detailed clinical and pathological data into sequencing studies have been limited (Table 4). Hence, the full clinical utility of genetic testing in veterinary oncology is still to be discovered.

Conclusion

The field of canine cancer genetics has been moving rapidly in recent years. We are in a position where we have tools and resources available to improve the

characterization of cancer in dogs. There are several examples of genetic-based tests entering the veterinary diagnostic market focused on cancer patients [67, 103]. This is a rapidly developing field with a large commercial market, yet we still lack clear clinical implications for how best to use these technologies to support clinical work. Continuous close collaboration between veterinary oncologists, pathologists and geneticists will guide the way for how we can use these technologies to benefit our patient population the most.

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