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# Comparative genomics reveals shared mutational landscape in canine hemangiosarcoma and human angiosarcoma

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Data availability

The whole-exome sequencing data, as well as the RNA-seq data that has not been previously published, has been submitted to the NCBI Sequence Read Archive (SRA). WES data: BioProject PRJNA552034, BioSamples SAMN12173468 - SAMN12173561. RNA-seq data: BioProject PRJNA562916, BioSamples SAMN12659339 - SAMN12659361.

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## Abstract

Angiosarcoma is a highly aggressive cancer of blood vessel-forming cells with few effective treatment options and high patient mortality. It is both rare and heterogenous, making large, well powered genomic studies nearly impossible. Dogs commonly suffer from a similar cancer, called hemangiosarcoma, with breeds like the golden retriever carrying heritable genetic factors that put them at high risk. If the clinical similarity of canine hemangiosarcoma and human angiosarcoma reflects shared genomic etiology, dogs could be a critically needed model for advancing angiosarcoma research. We assessed the genomic landscape of canine hemangiosarcoma via whole exome sequencing (47 golden retriever hemangiosarcomas) and RNA sequencing (74 hemangiosarcomas from multiple breeds). Somatic coding mutations occurred most frequently in the tumor suppressor *TP53* (59.6% of cases) as well as two genes in the PI3K pathway: the oncogene *PIK3CA* (29.8%) and its regulatory subunit *PIK3R1* (8.5%). The predominant mutational signature was the age-associated deamination of cytosine to thymine. As reported in human angiosarcoma, *CDKN2A/B* was recurrently deleted and *VEGFA, KDR*, and *KIT* recurrently gained. We compared the canine data to human data recently released by The Angiosarcoma Project, and found many of the same genes and pathways significantly enriched for somatic mutations, particularly in breast and visceral angiosarcomas. Canine hemangiosarcoma closely models the genomic landscape of human angiosarcoma of the breast and viscera, and is a powerful tool for investigating the pathogenesis of this devastating disease.

## Introduction

Angiosarcoma is an aggressive cancer of blood-vessel forming cells, associated with poor survival times (1–3). There is an unmet need for new diagnostics and therapies for these patients. However, the rarity of this cancer (approximately 0.01% of all cancers) (4,5) has limited large-scale genomic studies so far. Canine hemangiosarcoma is a relevant clinical model for understanding the pathophysiology of human angiosarcoma. The human and canine diseases share many clinical similarities, and hemangiosarcoma is common in dogs, occurring in some breeds (notably the golden retriever) with a frequency up to 20% (6). This means that using canine hemangiosarcoma as a model for human disease would yield sample cohorts of a magnitude inaccessible using human data alone. However, for dogs to be an effective model of this disease in the era of precision medicine, detailed genomic characterization of canine hemangiosarcoma must be undertaken, and the results directly compared to existing and emerging genomic data from human angiosarcoma.

Angiosarcoma can form anywhere in the vasculature. In human patients, it most commonly occurs in the skin of the head, neck, and scalp, the breast, the extremities, and less frequently

in the liver, right auricle of the heart, bone, and spleen (7). Prognosis is poor, with metastatic disease occurring in approximately 50% of cases (8), and a median overall survival time of approximately 50 months for local disease and 10 months in metastatic cases (9). Treatment involves surgical resection with wide margins, plus or minus radiation therapy, as well as adjuvant chemotherapy in the metastatic disease setting (3). Many angiosarcomas are initially sensitive to doxorubicin, paclitaxel, or targeted agents, but resistance to these therapies is virtually inevitable (3).

While most cases of angiosarcoma in humans occur without known cause, there are several known risk factors. These tumors can arise secondary to radiation therapy for other cancers or chronic lymphedema (3). Other known risk factors include UV irradiation, given the typical locations of cutaneous angiosarcomas on the head and neck (10–12), as well as occupational exposure to vinyl chloride (13), arsenic exposure (14), and use of anabolic steroids (15). Genetically, angiosarcoma is associated with familial syndromes including Li-Fraumeni syndrome (*TP53* mutations) (16) and Klippel-Trenaunay syndrome (*PIK3CA* mutations) (17). However, these syndromes do not solely cause angiosarcoma (7), and angiosarcomas are not present in the majority of cases.

Canine hemangiosarcoma is the histopathological equivalent of human angiosarcoma (18), and follows a similar, aggressive clinical course. In dogs, the most common tumor locations are the spleen, right auricle of the heart, liver, and skin or subcutaneous tissue (19,20). The different anatomical distribution seen in the human and canine diseases is likely due, at least in part, to the lack of secondary cases in dogs. Treatment protocols for dogs with hemangiosarcoma similarly involve wide surgical resection, followed by adjuvant chemotherapy. Survival times are short - a median of 4–6 months after surgical resection with adjuvant chemotherapy, with a 1 year survival rate of approximately 10% (21–23). Biological risk factors have not yet been identified in dogs. Genetically, dog breeds display differential predisposition to specific cancers, indicating that there are heritable risk factors that have become common as a result of inbreeding based on selection or drift. In a previous genome-wide association study, we identified several loci significantly associated with the risk of hemangiosarcoma in the golden retriever (24).

Recent targeted next-generation sequencing of human angiosarcomas has begun to reveal the somatic mutational spectrum of this disease. It has so far proven to be fairly heterogeneous - no pathognomonic mutations or copy number aberrations occur in all cases, and tumors from different primary locations or with different underlying etiologies have genomic differences. *TP53* and genes in the MAPK pathway are frequently mutated (25), and mutations in *PLCG1* and *PTPRB* are common, particularly in secondary angiosarcomas (25,26). Although the PI3K pathway is activated in some human angiosarcomas (27,28), *PIK3CA* mutations have so far not been commonly reported in human angiosarcoma studies (26,29). Genes in the VEGF pathway are frequently gained or amplified, including *VEGFA*, and *KDR* (25,28). The tumor suppressor *CDKN2A* is frequently deleted, while the *MYC* oncogene is frequently amplified, most commonly in radiation-associated tumors (25,28).

In dogs, a recent whole-exome sequencing study of a small cohort of 20 hemangiosarcomas showed that the top recurrently mutated genes were *PIK3CA* and *TP53* (30). Earlier

candidate gene studies of canine hemangiosarcomas reported mutations in *TP53* (31), *PTEN* (32), and *PDGFRA* and *PDGFRB* (33). An analysis of somatic copy number aberrations in visceral hemangiosarcomas from five breeds found that *VEGFA* was recurrently gained, while *CDKN2A* was frequently deleted (34). *MYC* copy number gain was infrequent, likely reflective of the fact that secondary hemangiosarcomas are not seen in canine patients (34). While these earlier studies provide clues as to the genetic features of canine hemangiosarcoma, there is very little of the genome-wide data needed for comprehensive comparison to human angiosarcoma.

In order to assess the potential utility of hemangiosarcoma as a model for angiosarcoma at the molecular level, we performed the largest exome sequencing study of hemangiosarcoma to date, and complemented our exome data with oligonucleotide array comparative genomic hybridization (oaCGH) copy number data and RNA sequencing (RNA-seq) data in partially overlapping cohorts of hemangiosarcoma cases. We then performed comparative analyses of our results with those released by The Angiosarcoma Project direct-to-patient initiative. In this way, we have created a detailed genomic profile of this cancer in the golden retriever breed, and begun vetting this canine cancer as a comparative model for human angiosarcoma, and potentially other tumors.

## Materials and Methods

Additional Materials and Methods can be found in the Supplementary Materials and Methods, and a workflow in Figure S1.

### Canine hemangiosarcoma sample collection for exome sequencing

Samples were obtained as part of necessary diagnostic procedures with owner consent. DNA from tumor tissue and whole blood was collected from 47 golden retrievers with visceral hemangiosarcoma. Samples were collected from dogs referred for treatment at the University of Minnesota (UMN) Veterinary Medical Center and samples submitted to the Modiano laboratory for diagnostic assessment and/or use in research (n = 34), from cases seen at the North Carolina State University (NCSU) Veterinary Hospital (n = 8), or from diagnostic samples sent to Antech Diagnostics (n = 5). Cases where date of diagnosis was known (n = 36), were diagnosed between 2000 and 2014. Tumor samples were either frozen (n = 17), or formalin-fixed and paraffin-embedded (FFPE, n = 30, Table S1). Procedures involving animal use were approved by the Institutional Animal Care and Use Committees at the Broad Institute, UMN, or NCSU.

### Sample preparation

DNA was extracted and sequencing libraries prepared using the Kapa Hyper Prep Kit (Table S2). For 66 samples, additional cycles of PCR were required to obtain sufficient DNA for exome capture, while libraries from 28 samples were re-constructed from source DNA using the standard protocol (See SI).

### Exome capture

The Roche Nimblegen SeqCap-EZ capture canine exome (120705_CF3_Uppsala_Broad_EZ_HX1) was used for hybrid exome capture, following the manufacturer's protocol.

### Sequencing and read alignments

The barcoded exome-captured libraries were multiplexed in pools of 8, and sequenced on the Illumina HiSeq 2500 to a target depth of 60x in the tumor and 30x in the normal, reaching a mean depth of 78x in the tumor and 63x in the normal. Reads were aligned to the CanFam3.1 reference genome using BWA. PCR duplicate reads were flagged using the Picard tool MarkDuplicates. Following the Genome Analysis Toolkit (GATK) Best Practices, we then performed Base Quality Score Recalibration (BQSR) using a set of approximately 19 million known canine germline variant locations.

### Somatic variant calling

Somatic mutations were called using MuTect2, using the default settings with the addition of the --*dontUseSoftClippedBases* option, which was added to avoid calling a large number of artifactual indels in our FFPE-preserved samples (See SI). In order to further refine our set of somatic variant calls, and to avoid artifacts, we also called variants using the GATK4 version of Mutect2, and kept only the consensus of calls which passed in both the GATK3 and GATK4 versions. Both sets of calls were filtered using a "panel of normals" created using all 47 normal (germline) samples, as well as germline samples from previous studies of canine lymphoma, osteosarcoma, and melanoma. Calls were further filtered to exclude oxidation artifacts, and were removed if they overlapped locations with known germline variants. Variants were further filtered if the position had low coverage (defined as a read depth less than 20 in the normal, less than 40 in the tumor, or less than 4 alternate allele reads in the tumor), or had excessive read depth (greater or equal to mean read depth + 5 x standard deviation), to filter out potential alignment errors. Finally, variants with a median read position < 10 were filtered to remove potential artifacts.

### Variant annotation

Variants were annotated using the variant effect prediction program SnpEff v4.2. Where multiple effects were predicted for a single variant, the most damaging predicted effect was selected. Coding variants were analyzed using the *smg* function in Genome MuSiC 0.4 to determine which genes were significantly mutated above the background rate. The *merge-concurrent-muts* option was applied to count multiple mutations in the same gene within a sample as a single mutation, and a false discovery rate (FDR) threshold of 0.1 using the convolution test (CT) was applied.

### Canine mutational signature discovery

Mutational signatures, considering point mutations and their genomic context, were extracted using a Bayesian non-negative matrix factorization (NMF) algorithm. The discovered signatures were compared to known signatures in COSMIC (35), as well as those

reported in the literature for human angiosarcoma (36). The overall landscape of mutations was plotted for different groupings of samples using the SomaticSignatures package.

### RNA-sequencing of canine hemangiosarcomas

Seventy-four snap-frozen samples were obtained from seventy-three dogs with hemangiosarcoma (41 golden retrievers and 32 dogs from 13 other breeds or from mixed breeding)(Kim, *et al.*, manuscript in preparation). RNA-seq data from 51 of the hemangiosarcoma tissues had been published previously (37,38). RNA-seq libraries were generated as described previously (37).

### Somatic copy number detection in canine hemangiosarcomas

Analysis of somatic copy number aberrations (SCNAs) in our canine whole exome sequencing data was limited by the inclusion of both frozen and formalin-fixed (FFPE) samples. The FFPE samples appeared to have an increased number of amplifications and deletions when compared with the frozen samples, possibly as a result of DNA fragmentation during the fixation process (39). While we were able to partially account for this using GC correction (40), we were not able to completely remove the bias between the two groups (Figure S6). Hence, we instead used oaCGH data to interrogate copy number changes in a cohort of 69 golden retrievers with hemangiosarcoma tumors, of which 28 were also included in the exome sequencing cohort. SCNAs were called from oligonucleotide array comparative genomic hybridization (oaCGH), as described previously (34), using a ~180,000-feature microarray (Agilent Technologies) with approximately 26 kb resolution throughout the dog genome.

### Accessing human data from The Angiosarcoma Project for comparative analysis

We compared the somatic mutations and SCNAs present in our cohort of canine hemangiosarcomas to the results reported by The Angiosarcoma Project, a direct-to-patient sequencing project run by the Count Me In initiative (JoinCountMeIn.org). Somatic mutations and SCNAs derived from whole exome sequencing data from 48 samples from 36 patients were downloaded from CBioPortal, and somatic mutations classified as nonsense, missense, splice site, or splice region were kept for analysis. For our analysis, we selected only the annotated primary tumor site from each patient, including 30 tumors - 17 breast, five visceral (one each of right atrium, spleen, lung, abdomen, and bladder wall), and eight head, face, neck, and scalp (HFNS) tumors.

### Pathway analysis in canine and human data

Pathway analysis was performed on the canine and human mutational data using the DAVID Functional Annotation Tool. For the overall enrichment analyses, all genes with coding nonsynonymous, splice site, or splice region somatic mutations were used. Functional annotation charts were created for KEGG pathways using the default options, using the Benjamini-Hochberg method to control the false discovery rate. Analysis was performed independently for the canine data and the human data, mapping genes from both species to *Homo sapiens* reference to avoid confounding due to differences in gene annotation between

the species. Canine Ensembl gene IDs were mapped to their human 1:1 orthologs using Ensembl's BioMart (41).

## Results

### Simple somatic mutations

**TP53 and PIK3CA are commonly mutated in canine hemangiosarcomas—**The seven significantly mutated genes (SMGs) in the canine hemangiosarcomas contained well-known cancer genes, including *TP53*, as well as two genes in the PI3K pathway (Table 1, Figure 1). Tumor suppressor *TP53* was most frequently mutated (28/47 cases, 59.6%), with all 28 cases carrying at least one mutation affecting the DNA binding domain (Figure 2). Oncogene *PIK3CA* (14/47, 29.8%) and its regulatory subunit *PIK3R1 (*4/47, 8.5%) were both mutated. Ten of the fourteen cases with *PIK3CA* mutations had a mutation at amino acid position 1047, a hotspot frequently mutated in many types of human cancers (42) (Figure 2). The remaining four SMGs were *ORC1* (4/47, 8.5%), *RASA1* (4/47, 8.5%), *ARPC1A* (3/47, 6.4%), and *ENSCAFG00000017407* (2/47, 4.3%), a "one-to-many" ortholog of human *ATP5PD* with 27 paralogs in the canine genome. Overall, 59 genes that were mutated at least once in the canine dataset are annotated as likely causal in the COSMIC Cancer Gene Census (43), including *TP53*, *PIK3CA*, and *PIK3R1* (Table S3).

**Variant allele fraction and effect predictions—**For further insight into whether the four SMGs not annotated in COSMIC are likely to be driver mutations, we investigated the variant allele fraction (VAF) of each and whether missense mutations were predicted to be deleterious or tolerated using the SIFT (44) score via Ensembl's Variant Effect Predictor (45). The median VAF for the four *RASA1* mutations was 0.12 (range 0.08 – 0.23). Three mutations were nonsense mutations, with the fourth a missense mutation predicted to to be deleterious (SIFT score 0), supporting a causal role for these mutations. Similarly, the median VAF of the three *ARPC1A* mutations was 0.16 (range 0.12 – 0.17), with one nonsense mutation, and two missense mutations at the same position, predicted to be deleterious (SIFT score 0). The median VAF for the four *ORC1* mutations (all at the same position) was lower, at 0.08, and the change was predicted to be tolerated (SIFT score 0.06). The two mutations in *ENSCAFG00000017407* also had a low median VAF (0.05), and were predicted to be tolerated (SIFT score 1).

**Comparison with top mutated genes in human angiosarcoma—**The most commonly mutated genes in the human data were *TP53* (8/30 tumors, 29%), followed by *KDR, LRP2, RYR2*, and *ABCA13* with mutations in 7/30 tumors each, and *PIK3CA, FLG, ASXL3, MYH14*, and *UNC13C*, mutated in 6/30 tumors each. However, the distribution of these mutations varied by tumor location. In the human data, breast tumors were the only location to carry *PIK3CA* mutations (n = 6 patients), and were the most common location for *KDR* mutations. *TP53* mutations occurred in all locations, but were more common in HFNS and visceral tumors. *KDR* was much less frequently mutated in the canine cohort, with only one case having a nonsynonymous mutation, predicted to be tolerated. Of the canine SMGs, two human tumors also had *RASA1* mutations; one was a nonsense mutation and the other was predicted to be deleterious.

**Differential patterns of mutations found in some tumor locations—**The pattern of mutations in the PI3K gene family varied by tumor location in the canine data, as well. In the canine cases, a slightly higher proportion of heart tumors (11/16, 68.8%) compared to splenic tumors (9/22, 40.9%) had PI3K alterations, although this difference was not significant ($p_{chi\text{-}sq}$=0.09). Liver tumors had slightly fewer overall alterations in the PI3K gene family (3/8, 37.5%), and were less likely to have *PIK3CA* mutations (1/8, 12.5%), although this was not statistically significant.

**Comparison of mutational burden by tumor location in canine and human data—**We found fewer nonsynonymous mutations in the canine tumors than reported in the human tumors overall, while the number of nonsynonymous, splice site, or splice region mutations per sample varied significantly by tumor location in human cases. In the canine cohort, there were a median of 23 nonsynonymous coding mutations per sample (range 1–152; Supplementary Table S1), which was closest to the human breast (median = 32, n = 17), and visceral (median = 40, n = 5) tumors. In the dogs, there was a small difference in the total number of nonsynonymous and splice site/region mutations between heart (median = 31, n = 15, outlier with 152 mutations removed) and splenic (n = 22, median = 17.5) tumors ($p_{TukeyHSD}$ = 0.04). In the human data, there was a significant difference in the median number of mutations by tumor location, with head, face, neck, and scalp (HFNS, n = 8) tumors having a higher mutational burden (median = 596.5) than breast or visceral tumors ($p_{ANOVA} = 3.6 \times 10^{-5}$)(46).

**Comparative pathway analysis highlights similarity between canine and human tumors—**We compared the gene functional annotations enriched in hemangiosarcoma to those enriched in breast and visceral angiosarcoma and those enriched in HFNS angiosarcoma (Table 2). KEGG pathways enriched in canine hemangiosarcoma overlapped with KEGG pathways enriched in both subtypes of angiosarcoma. Pathways enriched in hemangiosarcoma included a larger fraction of those enriched in visceral and breast angiosarcoma (75%, vs. 48% of pathways enriched in HFNS angiosarcoma), however, the total number of pathways shared was greater between hemangiosarcoma and HFNS angiosarcoma (10 pathways). Pathways shared between hemangiosarcoma and visceral and breast angiosarcoma included pathways in cancer ($p_{canine} = 3.6 \times 10^{-4}$, $p_{visceral/breast} = 4.8 \times 10^{-2}$), Rap1 signaling pathway ($p_{canine} = 5.8 \times 10^{-3}$, $p_{visceral/breast} = 9.0 \times 10^{-3}$) and central carbon metabolism in cancer ($p_{canine} = 3.0 \times 10^{-3}$, $p_{visceral/breast} = 2.3 \times 10^{-2}$). Pathways shared between hemangiosarcoma, visceral and breast angiosarcoma, and HFNS angiosarcoma included focal adhesion pathway ($p_{canine} = 1.4 \times 10^{-3}$, $p_{visceral/breast} = 2.2 \times 10^{-2}$, $p_{HFNS} = 8.1 \times 10^{-4}$), calcium signaling pathway ($p_{canine} = 2.8 \times 10^{-2}$, $p_{visceral/breast} = 5.4 \times 10^{-3}$, $p_{HFNS} = 1.9 \times 10^{-2}$), and long-term depression ($p_{canine} = 1.5 \times 10^{-2}$, $p_{visceral/breast} = 7.1 \times 10^{-3}$, $p_{HFNS} = 8.6 \times 10^{-3}$).

In addition, functional annotation enrichment analysis of the genes mutated in hemangiosarcoma, visceral and breast angiosarcoma, and HFNS angiosarcoma found that they were enriched in many of the same protein domains. Shared domains include Fibronectin Type III ($p_{canine} = 9.5 \times 10^{-9}$, $p_{visceral/breast} = 6.3 \times 10^{-5}$, $p_{HFNS} = 3.0 \times 10^{-15}$), Epidermal growth factor-like domain ($p_{canine} = 1.5 \times 10^{-3}$, $p_{visceral/breast} = 5.6 \times 10^{-5}$, $p_{HFNS}$

= $4.0 \times 10^{-16}$), and Tyrosine protein kinase active site ($p_{canine} = 6.9 \times 10^{-4}$, $p_{visceral/breast} = 3.8 \times 10^{-3}$, $p_{HFNS} = 2.3 \times 10^{-6}$). Both visceral and breast and HFNS angiosarcomas were enriched in cadherins ($p_{visceral/breast} = 4.9 \times 10^{-4}$, $p_{HFNS} = 4.7 \times 10^{-16}$), HFNS highly so, while hemangiosarcoma was not ($p_{canine} = 0.14$). In addition, HFNS angiosarcoma was enriched in ABC-transporters ($p = 2.8 \times 10^{-7}$), while visceral and breast angiosarcoma and canine hemangiosarcoma were not (Table S7).

**Pathways and gene families previously reported in the angiosarcoma literature—**Several pathways and gene families have been previously reported to be affected in the human angiosarcoma literature. Mutations in both *PLCG1 and PTPRB* have been reported to be recurrent (26), particularly in secondary angiosarcoma. In the human data, 12 patients had mutations in eight phospholipase C genes, including *PLCG1* (n = 5, Table 3). Seven canine cases (15%) had mutations in PLC genes, including one in *PLCG1*. Twenty protein tyrosine phosphatases were mutated in twelve samples in human, including two patients with *PTPRB* mutations. While none of the canine tumors had somatic mutations in *PTPRB*, eight cases (17%) harbored a mutation in one of nine other protein tyrosine phosphatase genes (Table 3).

The MAPK pathway has been reported to be frequently affected by mutations in angiosarcoma (25), and we found the visceral and breast angiosarcomas to be enriched in mutations in MAPK pathway genes (p = 0.02, Table 2). We noted mutations in 25 genes in the MAPK pathway in the canine cohort, including the SMGs *TP53* and *RASA1*. In the human data, 67 genes in the MAPK pathway were mutated. Thirteen of these genes were mutated in both the canine and human data (Table 3). Although we found mutations in genes involved in histone methyltransferase/demethylase activity in both species, consistent with earlier human studies (47), we saw no significant enrichment on a pathway level in either the canine or human cohorts (Table 3). Tyrosine protein kinases were enriched in both hemangiosarcoma and angiosarcoma. In the human data, 55 genes in the GO Protein Tyrosine Kinase Activity pathway were mutated, while 22 genes in this pathway were mutated in the canine data, with 12 genes mutated in both human and canine samples (Table 3). Low-density lipoprotein receptors were enriched for mutations in the canine data (p = 0.03), and human HFNS tumors (p = 0.05), and *LRP2* was one of the most frequently mutated genes in the human dataset (mutated in 7/30 tumors). While mutations in the LDL receptor-related protein family have not been widely reported in human angiosarcoma, they have recently been shown to play a role in a variety of cancers (48). We found that four LDL receptor-related protein genes were mutated in both hemangiosarcoma and angiosarcoma, with an additional gene mutated in angiosarcoma only (Table 3).

**Mutational signature of aging is present across all canine tumors—**Analysis of mutational signatures, which capture the mutational landscape of tumors and are shaped by both genetic factors and environmental exposure, revealed similarities between canine hemangiosarcoma and human angiosarcoma, as reported by Thibodeau, *et al.* (36). We analyzed the signatures of trinucleotide mutational frequencies present in golden retriever hemangiosarcomas, and found a strong signature of mutations arising through spontaneous deamination of methylated cytosines in CpG islands, corresponding to COSMIC signature

SBS1 and generally associated with aging (35) (Figure 3, Figures S3–S5). In addition, we see a faint signature not described in COSMIC (Figure 3). The overall mutational landscape was consistent across all canine hemangiosarcoma tumors, regardless of tumor location or tissue preservation method (Figures S2 – S4).

**Canine RNA-seq analysis validates exome mutations and extends findings to additional breeds—**We validated our exome sequencing somatic mutation calls using RNA-seq data from a partially overlapping set of dogs (n = 74 tumor samples from 73 dogs from 14 breeds, as well as mixed-breed dogs, Table S4). Thirteen golden retrievers from the exome cohort with mutations in SMGs had the same tumor location included in the RNA-seq data, with a total of 25 SMG mutations among them. We excluded 2 frameshift deletion variants from the validation, as they showed evidence of deletion in the pileup data, but also unexpected alternate alleles. Of the remaining 23 variants, we validated 15 (65%) mutations. Of the eight sites which were not validated, one was a nonsense mutation, which may have been subject to nonsense-mediated decay, five had a variant allele fraction (VAF) less than 0.1, and three variants came from samples with tumor purity estimate (≤ 35%) on histological examination, suggesting that tumor heterogeneity and stromal contamination may be a contributing factor to variants not being replicated (Table S5). In the RNA-seq data, four tumor samples from three dogs with variants in the SMGs were from a different tumor site than was included in the exome discovery cohort. In this subset, we were able to detect the mutation discovered in the same individual, but a different tumor location in 4/8 (50%) cases. Overall, the group of variants which we were unable to validate came from tumor samples with a significantly lower estimated tumor purity on histology ($p_{t\text{-}test} = 0.04$), but this difference was not significant based on tumor purity estimates from the program ESTIMATE ($p_{t\text{-}test} = 0.4$, Table S5).

The RNA-seq cohort also confirmed that the *TP53* and PI3K mutations were not breed-specific, but were present across different breeds. Excluding the 19 golden retrievers who were also included in the exome cohort, we looked to see whether somatic mutations corresponding to those observed in the exome cohort could be found in other cases, which included golden retrievers (n = 22), Portuguese water dogs (PWD, n = 6), German Shepherd Dogs (GSD, n = 6), mixes (n = 6), boxers (n = 2), Labrador retrievers (Labs, n = 2), Keeshonds (n = 2), and other breeds (n = 8, one each of American Staffordshire Terrier, Bernese Mountain Dog, Bichon Frise, Briard, Bullmastiff, Gordon setter, Parson's Russell Terrier, and Saluki). We found mutations at the same sites as discovered in the exome cohort in *TP53* (n = 14, 26%, 5 breeds), *PIK3CA* (n = 11, 20%, 6 breeds), and *PIK3R1* (n = 4, 7%, 4 breeds, Table S6).

## Somatic copy number aberrations

**SCNAs in canine hemangiosarcoma recurrently affect known cancer genes—**We surveyed SCNAs in genes known to be involved in hemangiosarcoma and angiosarcoma. The genes most recurrently affected by DNA copy number aberrations in the oaCGH data were *VEGFA*, with copy number gain in 19% of cases, *KDR*, gained in 22%, *KIT*, gained in 17%, and the tumor suppressor *CDKN2A/B*, deleted in 22% (Table S8). The *MYC*

oncogene had copy number gain in 9% of cases. Copy number aberrations in the top significantly mutated genes were relatively rare (Figure 1A, Table S8).

**Copy number gains in KDR and losses in AXIN1 are common in both dogs and humans—**Comparison of SCNAs in the human data and canine oaCGH data revealed recurrent copy number aberrations in known cancer genes *KDR* and *AXIN1* in both species. Copy number gains in *KDR* occurred in approximately 27% of human samples, and 22% of canine samples. *AXIN1* was lost in 20% of human and 22% of canine samples. In addition, the genes *PTK6*, *ARFRP1*, and *RTEL1* showed copy number loss in 17% of human and canine samples, however, these genes are close together, and also showed copy number gains in a number of canine samples (Table S9).

**SCNA profiles differ among cases with and without PIK3CA mutations—**We examined the SCNA profiles of hemangiosarcoma cases with and without *TP53* and *PIK3CA* mutations in the 28 cases with both exome sequencing and oaCGH data. There were no significant differences in the relative frequency of any given CNA between the CNA profiles of cases with and without *TP53* mutations. However, significant differences were detected between cases with *PIK3CA* mutations and those without (Figure 4), using a two-tailed Fisher's Exact test and a minimum differential threshold of 25% between the two groups. A region on chromosome 11 at approximately 22.6 Mb, near the *UBE2B* (ubiquitin-conjugating enzyme E2B) gene, was deleted in 4/11 cases with *PIK3CA* mutations, and 0/17 without ($p < 0.016$). Similarly, the *CDKN2B* gene, located distally on chromosome 11 at 41.2Mb, was deleted in 4/11 cases with *PIK3CA* mutations, and 0/17 cases without ($p < 0.016$). A region on chromosome 24 at 21.2 Mb was gained in 7/17 cases without *PIK3CA* mutation and 0/11 with *PIK3CA* mutation ($p < 0.023$). This region overlaps the anti-apoptotic *BCL2L1* gene. Broad copy number gains along the length of chromosome 31 were more frequent in cases without *PIK3CA* mutations compared to those with *PIK3CA* mutations.

## Discussion

Detailed molecular profiling of canine hemangiosarcoma has revealed both similarities and differences in the genetic landscape between dogs and humans. In particular, visceral canine hemangiosarcoma showed strong similarities to human angiosarcoma of the viscera and breast. Our findings have important implications for comparative oncology, as the study of canine hemangiosarcoma has the potential to improve our understanding of the pathophysiology of both canine hemangiosarcoma and human angiosarcoma, and to improve treatment and outcomes in both species.

Tumor suppressor *TP53* was the top significantly mutated gene in the canine data. The majority of mutations occurred in the DNA binding domain, likely causing loss of function (Figure 2). *TP53* was also the only significantly mutated gene in the human Angiosarcoma Project data, and it has been frequently reported as mutated in targeted sequencing studies of angiosarcoma (25).

We found that the PI3K pathway was commonly mutated in canine hemangiosarcoma. A total of 23 canine tumors (48.9%) had at least one somatic mutation affecting this gene family (Figure 1A). Tumors with a mutation in the PI3K family tended to have only one mutation in this family. The PI3K pathway is one of the most commonly altered pathways in cancer, playing an important role in signal transduction leading to cell proliferation, survival, differentiation, and regulation of metabolism and immunity (49,50). *PIK3CA* is an oncogene (51) that has been shown to be mutated in human glioblastoma, breast, gastric, colorectal, lung, and endometrial cancers (52). Ten of the 14 *PIK3CA* mutations in our canine cohort occurred at amino acid position 1047, a mutational hotspot in many human cancers (42). Mutations within this domain have been shown to increase catalytic activity (53).

Of the less frequently mutated SMGs, *PIK3R1* is annotated as a likely driver mutation by COSMIC. In addition, variant allele fraction and SIFT scores support a potential role for *RASA1* and *ARPC1A* as driver mutations. *RASA1* is a negative regulator of the RAS and MAPK pathways, and plays an important role in vascular formation (54,55). Germline *RASA1* mutations can cause capillary malformation - arteriovenous malformation syndrome (56). Somatic mutations in this gene have been found in a subset of human basal cell carcinomas, and expression has been correlated with survival in invasive ductal breast carcinomas and hepatocellular carcinomas (57,58). *ARPC1A* plays an important role in regulating the actin cytoskeleton, which functions in the migration and invasiveness of pancreatic carcinoma cells (59). There is less support for a causal role for *ORC1* and *ENSCAFG00000017407*, however, it is important to note that the SIFT score does not annotate activating mutations - for example, the hotspot mutations in *PIK3CA* (known to be drivers in many human cancers) are also predicted to be tolerated.

There were also potentially important differences in somatic mutations between the two species. In the human data, mutations in *TP53* and *PIK3CA* tended to be mutually exclusive, while we did not see this pattern in the canine tumors. In addition, *PIK3CA* mutations were exclusively found in breast tumors in the small human data set, while we found them to be common in cardiac and splenic tumors in dogs. Within the canine visceral tumors, we found fewer mutations in *PIK3CA* in liver tumors. These differences in distribution of *PIK3CA* mutations by tumor location may be due to genetic heterogeneity of the cancer, with tumors in different locations activating the PI3K pathway at different points, or relying on alterations in different pathways to affect an essential protein downstream. It is also possible that this difference is due to the small number of human visceral angiosarcomas currently sequenced, and the small number of liver tumors in the canine cohort.

Another potentially important difference between the two species is that, while copy number gains in *KDR* are common in both species, somatic mutations in this gene were seen in over 20% of human tumors, but in only one canine tumor. As the *KDR* receptor is upstream of the PI3K pathway, it is possible that mutations in either may lead to a similar phenotype. We also saw more copy number gains of *VEGFA* in our canine cohort (19% vs. 0 in the human data), and as *VEGFA* is upstream of *KDR*, this copy number gain may serve a similar role to *KDR* mutations in the canine tumors. In addition, whole genome sequencing will be necessary to determine whether there are common regulatory mutations affecting this gene in canine hemangiosarcoma.

In the human data, mutation rates were significantly different between different tumor locations, with head and neck angiosarcomas having a much higher mutational burden, as reported by Painter, *et al.* (46). It is possible that the higher mutational burden in these tumors is due to UV exposure. It would be interesting to compare these findings to canine cutaneous and subcutaneous hemangiosarcomas, which were not included in the current study. In the canine visceral hemangiosarcoma, a slight difference was found between mutational burden in heart and splenic tumors. It is possible that the golden retrievers have a lower overall somatic mutation burden than humans, as they likely have a higher germline risk burden, given the high incidence of hemangiosarcoma in the breed. Further studies in dogs will be necessary to determine whether the lower mutation rate is correlated with breed risk, or if in this case it is an artifact of how mutations were called.

Tumors in both dogs and humans were enriched for mutations in protein tyrosine kinases, which are important regulators of cellular growth and division signals and are commonly mutated in cancers. There were also recurrent mutations in the protein tyrosine phosphatase gene family in both species. This may suggest an alternate mechanism of tyrosine kinase overactivation, as protein tyrosine phosphatases deactivate tyrosine kinase signaling by dephosphorylating proteins in opposition to kinase phosphorylation (60). In addition, phospholipase C proteins play a crucial role in cellular signalling pathways by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messengers DAG and IP3, passing on signals from receptor tyrosine kinases (61). The canine hemangiosarcomas were enriched for phospholipase C genes, including *PLCG1*, and *PLCG1* mutations were recurrent in the human tumors.

The shared enriched pathways between canine hemangiosarcomas and human angiosarcomas provide insight into disease pathogenesis for both species. Tyrosine kinase inhibitors have been effective against angiosarcoma in the clinic, but tumor heterogeneity and the development of resistance have limited their long-term utility. They have also shown promise against hemangiosarcoma *in vitro* (62), but so far have been less promising in the veterinary clinic (63). Future investigation of the interaction between the many affected pathways will help to determine the potential for combination therapy targeting multiple of these pathways or a common downstream effector to combat resistance.

A recent study in 13 radiation-induced and 3 spontaneous breast angiosarcomas detected the irradiation signature, as well as the aging signature, and a unique C>T signature (36). The median age of patients in this human cohort was 74.5 years (36). Canine hemangiosarcomas looked very similar, in that we primarily saw the aging signature and low levels of a novel C>T signature. This novel signature bore some resemblance to the signature reported in the human angiosarcomas, including higher levels of C>T mutations at C nucleotides flanked by A-A or A-T, however, there were also differences, such as a high number of mutations flanked by T-G in the dogs, and a low number of these in the human cohort. A larger study will be needed to decipher whether this is a novel angiosarcoma-related signature or whether it represents noise. The lack of the irradiation signature was anticipated as our canine data did not include any tumors secondary to radiation therapy.

We examined copy number changes in canine hemangiosarcoma in genes previously reported to be affected in hemangiosarcoma and angiosarcoma. Most common were copy number gain of *VEGFA* and *KDR*, and loss of *CDKN2A/B*. The *MYC* oncogene, which has been reported amplified in human angiosarcoma and canine hemangiosarcoma, was only rarely gained in our dataset and showed no evidence of high-level amplification. This makes sense, given that it is more common in radiation-induced tumors, which were not present in the canine cohort.

Our data suggest that visceral canine hemangiosarcoma could be developed as a model for primary human angiosarcoma. Detailed molecular characterization of canine hemangiosarcoma revealed many similarities, but also some important differences, between canine hemangiosarcoma and human angiosarcoma. Future work should include analysis of larger sample sizes, including recalling all currently available human angiosarcoma data using the same methods, in order to decipher potential molecular subtypes and to facilitate a more complete comparison between tumors in different locations. An integrated understanding of the interaction between mutations in the many enriched signaling pathways may be useful for determining treatment strategy, for example, the feasibility of combinations of targeted inhibitors or the prevention of convergent resistance. Our data suggest that clinical trials evaluating therapeutic approaches in dogs with this disease might also inform human medicine.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Implications

We characterize the genomic landscape of canine hemangiosarcoma and demonstrate its similarity to human angiosarcoma.

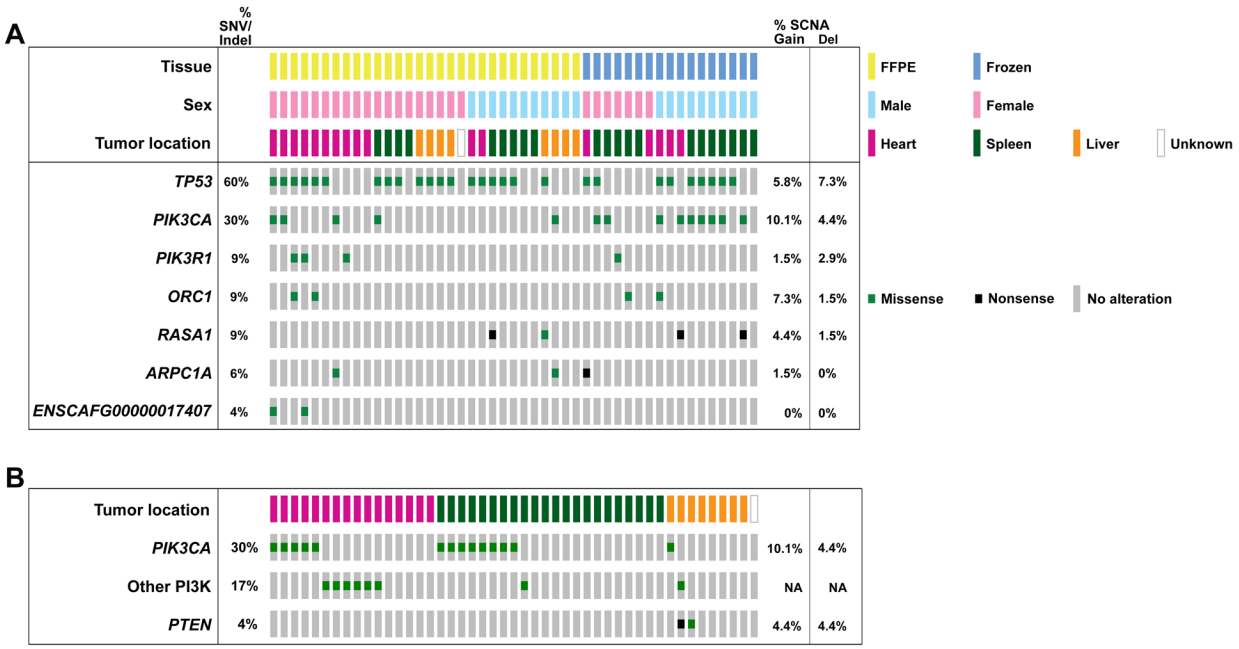


**Figure 1. Per-sample annotation of metadata.**

A) somatic mutations in the seven significantly mutated genes B) somatic mutations in the PI3K gene family by tumor location. 'Other PI3K' category includes mutations in PIK3CB, PIK3C2G, PIK3C3, PIK3R1, and PIK3R5). SNV, single nucleotide variant; Indel, insertion/deletion; SCNA, somatic copy number aberrations. SCNA frequencies are calculated based on oaCGH data from 69 canine hemangiosarcoma samples.

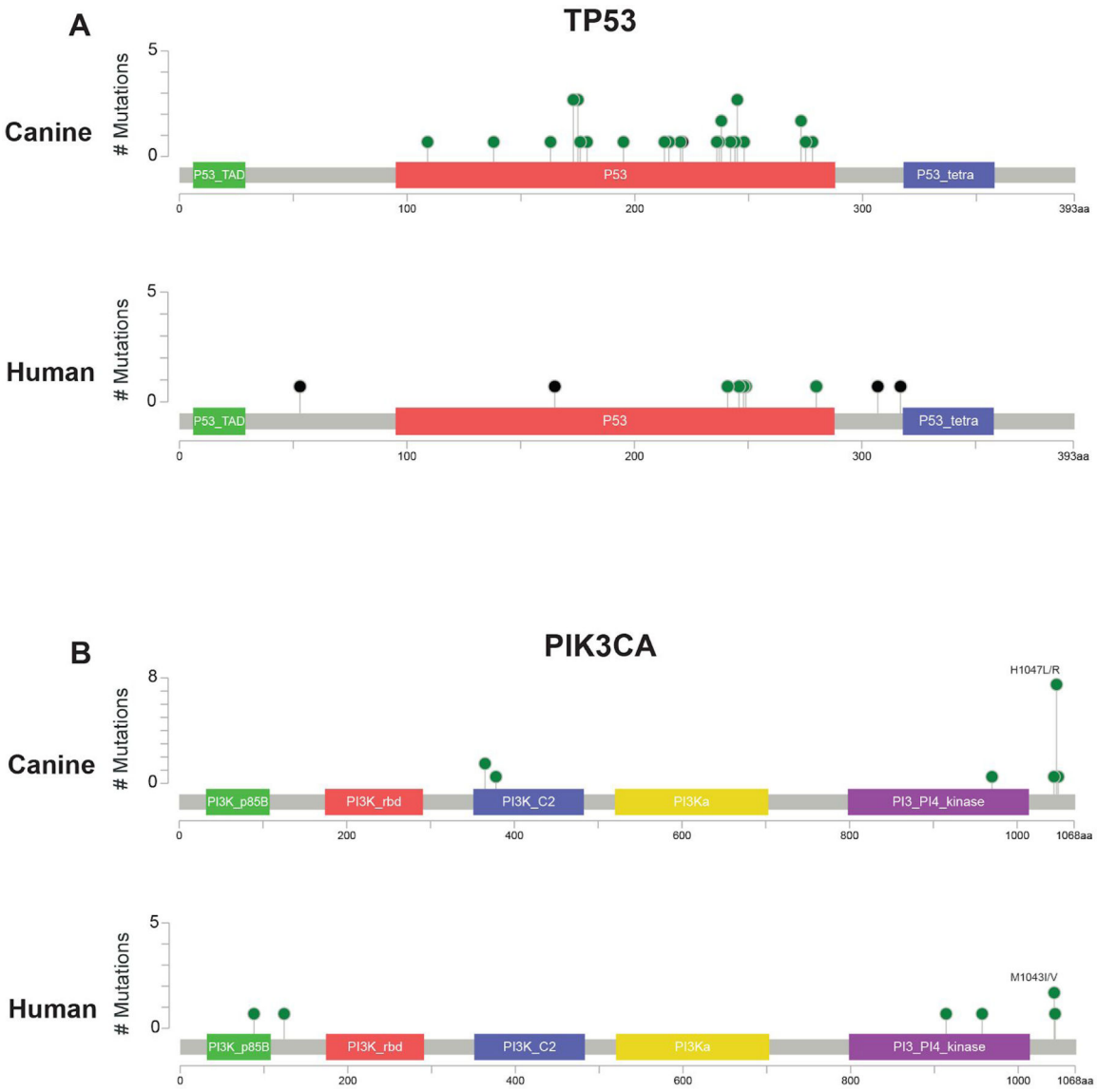


**Figure 2.**

Lollipop plots showing the positions of the (A) Tumor protein p53 (TP53) and (B) Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutations in the canine and human data. Canine mutational locations were lifted over to the human hg19 reference genome using the LiftOver tool. Green: missense mutation, Black: truncating mutation.

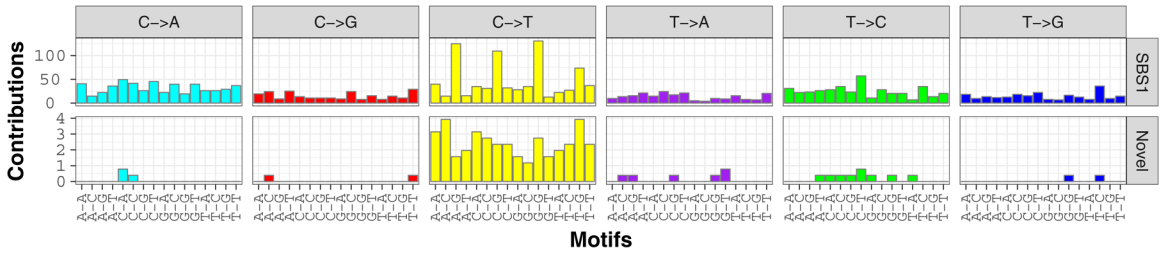


**Figure 3.**

Mutational signatures called using Bayesian NMF in the entire canine hemangiosarcoma cohort, showing the count of mutations (y-axis) in each trinucleotide context (x-axis). This analysis reveals the COSMIC SBS1 aging signature (top), and a faint novel signature (bottom).

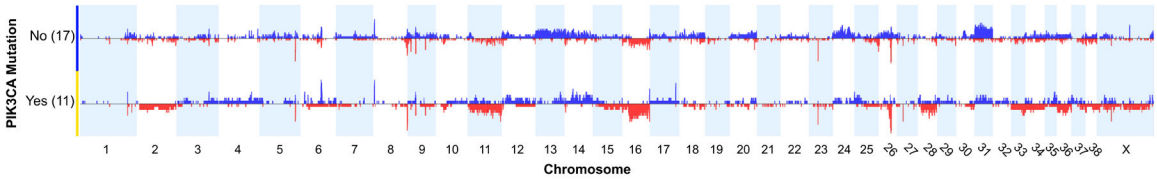


**Figure 4.**

Comparison of DNA copy number aberration profiles between cases with *PIK3CA* mutation and those without. Blue = copy number gain, red = copy number loss. (Thomas, *et al.*, manuscript in preparation)(34)

**Table 1.**

Significantly mutated genes in golden retriever hemangiosarcoma tumors. Significantly mutated genes with an FDR < 0.1, calculated using Genome MuSiC. SNVs: single nucleotide variants, Covd Bps: number of basepairs with adequate coverage in gene, Muts pMbp: mutations per megabase.

| Gene ID | Gene name | Indels | SNVs | Total Mutations | Covd Bps | Muts pMbp | p-value | FDR |
|---|---|---|---|---|---|---|---|---|
| ***TP53*** | Tumor protein p53 | 3 | 25 | 28 | 72,582 | 386 | 0 | 0 |
| ***PIK3CA*** | Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha | 0 | 14 | 14 | 156,415 | 90 | 0 | 0 |
| ***PIK3R1*** | phosphoinositide-3-kinase regulatory subunit 1 | 0 | 4 | 4 | 124,857 | 32 | $7.3\times10^{-8}$ | $5.9\times10^{-4}$ |
| ***ORC1*** | origin recognition complex subunit 1 | 0 | 4 | 4 | 133,219 | 30 | $1.3\times10^{-7}$ | $8.2\times10^{-4}$ |
| ***RASA1*** | RAS p21 protein activator 1 | 0 | 4 | 4 | 136,539 | 29 | $3.2\times10^{-6}$ | $1.6\times10^{-2}$ |
| ***ARPC1A*** | actin related protein 2/3 complex subunit 1A | 0 | 3 | 3 | 72,351 | 41 | $1.3\times10^{-5}$ | $5.1\times10^{-2}$ |
| ***ENSCAFG00000017407*** | 1-to-many ortholog of human ATP synthase peripheral stalk subunit d (*ATP5PD*) | 0 | 2 | 2 | 22,074 | 91 | $1.7\times10^{-5}$ | $5.9\times10^{-2}$ |

**Table 2.**

KEGG pathways enriched in hemangiosarcoma, breast and visceral angiosarcoma, and HFNS angiosarcoma. Blue: pathways enriched in both visceral and breast angiosarcoma and hemangiosarcoma; Yellow: pathways enriched in both HFNS angiosarcoma and hemangiosarcoma; Green: pathways enriched in all three groups.

| Angiosarcoma - visceral and breast | | Hemangiosarcoma | | Angiosarcoma - HFNS | |
|---|---|---|---|---|---|
| **Term** | **p-value** | **Term** | **p-value** | **Term** | **p-value** |
| hsa04020:Calcium signaling pathway | 5.4E-03 | hsa05200:Pathways in cancer | 3.6E-04 | hsa02010:ABC transporters | 2.5E-08 |
| hsa04730:Long-term depression | 7.1E-03 | hsa04360:Axon guidance | 3.8E-04 | hsa04510:Focal adhesion | 8.1E-04 |
| hsa04015:Rap1 signaling pathway | 9.0E-03 | hsa04919:Thyroid hormone signaling pathway | 5.9E-04 | hsa04512:ECM-receptor interaction | 9.6E-04 |
| hsa04510:Focal adhesion | 2.2E-02 | hsa04664:Fc epsilon RI signaling pathway | 6.7E-04 | hsa04740:Olfactory transduction | 1.1E-03 |
| hsa04720:Long-term potentiation | 2.2E-02 | hsa04510:Focal adhesion | 1.4E-03 | hsa04724:Glutamatergic synapse | 1.3E-03 |
| hsa04010:MAPK signaling pathway | 2.3E-02 | hsa05146:Amoebiasis | 1.4E-03 | hsa04974:Protein digestion and absorption | 2.9E-03 |
| hsa05230:Central carbon metabolism in cancer | 2.3E-02 | hsa05214:Glioma | 1.5E-03 | hsa04611:Platelet activation | 3.8E-03 |
| hsa05200:Pathways in cancer | 4.8E-02 | hsa05215:Prostate cancer | 1.5E-03 | hsa04610:Complement and coagulation cascades | 8.1E-03 |
| | | hsa04070:Phosphatidylinositol signaling system | 1.7E-03 | hsa04976:Bile secretion | 8.1E-03 |
| | | hsa05230:Central carbon metabolism in cancer | 3.0E-03 | hsa04730:Long-term depression | 8.6E-03 |
| | | hsa05223:Non-small cell lung cancer | 3.4E-03 | hsa04713:Circadian entrainment | 8.7E-03 |
| | | hsa05221:Acute myeloid leukemia | 3.4E-03 | hsa04723:Retrograde endocannabinoid signaling | 9.5E-03 |
| | | hsa04750:Inflammatory mediator regulation of TRP channels | 3.8E-03 | hsa04022:cGMP-PKG signaling pathway | 1.8E-02 |
| | | hsa04012:ErbB signaling pathway | 3.9E-03 | hsa05033:Nicotine addiction | 1.9E-02 |
| | | hsa04015:Rap1 signaling pathway | 5.8E-03 | hsa04020:Calcium signaling pathway | 1.9E-02 |
| | | hsa05213:Endometrial cancer | 5.9E-03 | hsa04024:cAMP signaling pathway | 2.9E-02 |
| | | hsa04151:PI3K-Akt signaling pathway | 1.2E-02 | hsa05146:Amoebiasis | 3.4E-02 |
| | | hsa05231:Choline metabolism in cancer | 1.3E-02 | hsa04151:PI3K-Akt signaling pathway | 4.1E-02 |
| | | hsa04014:Ras signaling pathway | 1.3E-02 | hsa04080:Neuroactive ligand-receptor interaction | 4.4E-02 |
| | | hsa04730:Long-term depression | 1.5E-02 | hsa05205:Proteoglycans in cancer | 4.6E-02 |
| | | hsa00562:Inositol phosphate metabolism | 1.5E-02 | hsa04925:Aldosterone synthesis and secretion | 4.8E-02 |
| | | hsa04370:VEGF signaling pathway | 1.6E-02 | | |
| | | hsa04713:Circadian entrainment | 1.7E-02 | | |

| Angiosarcoma - visceral and breast | | | Hemangiosarcoma | | | Angiosarcoma - HFNS | |
|---|---|---|---|---|---|---|---|
| Term | p-value | | Term | p-value | | Term | p-value |
| | | | hsa05222:Small cell lung cancer | 1.9E-02 | | | |
| | | | hsa04725:Cholinergic synapse | 2.2E-02 | | | |
| | | | hsa04915:Estrogen signaling pathway | 2.3E-02 | | | |
| | | | hsa04723:Retrograde endocannabinoid signaling | 2.5E-02 | | | |
| | | | hsa04724:Glutamatergic synapse | 2.6E-02 | | | |
| | | | hsa04020:Calcium signaling pathway | 2.8E-02 | | | |
| | | | hsa04662:B cell receptor signaling pathway | 2.9E-02 | | | |
| | | | hsa04611:Platelet activation | 3.1E-02 | | | |
| | | | hsa05218:Melanoma | 3.3E-02 | | | |
| | | | hsa04917:Prolactin signaling pathway | 3.3E-02 | | | |
| | | | hsa05220:Chronic myeloid leukemia | 3.6E-02 | | | |
| | | | hsa05205:Proteoglycans in cancer | 3.7E-02 | | | |
| | | | hsa04921:Oxytocin signaling pathway | 4.5E-02 | | | |

**Table 3.**

Overlap of mutated genes between canine hemangiosarcoma and human angiosarcoma tumors in several gene families and pathways. Significantly mutated genes bolded.

| Gene family | Human only | Shared | Canine only |
|---|---|---|---|
| **Phosphatidyl- inositol signaling** KEGG Pathway | *DGKB, DGKD, DGKQ, IMPA2, INPP4B, ITPR1, ITPR2, ITPR3, PI4KA, PIK3CG, PIK3R2, PIP5K1B, PLCB1, PRKCB* | *DGKI, PIK3C2G,* ***PIK3CA****, PLCB2, PLCB3, PLCG1, PLCG2, PTEN* | *DGKG, PIK3C3, PIK3CB,* ***PIK3R1****, PIK3R5, PLCB4, PLCE1* |
| **Phospholipase C** | *PLCB1, PLCH1, PLCXD1, PLCXD3* | *PLCB2, PLCB3, PLCG1, PLCG2* | *PLCB4, PLCE1* |
| **Protein tyrosine phosphatase** | *PTP4A2, PTPN7, PTPN9, PTPN13, PTPN23, PTPRA, PTPRB, PTPRC, PTPRH, PTPRO, PTPRQ, PTPRR, PTPRS, PTPRT* | *PTPN5, PTPN22, PTPRD, PTPRJ, PTPRK, PTPRZ1* | *PTP4A1, PTPDC1, PTPN4* |
| **LDL receptor related proteins** | *LRP3* | *LRP1, LRP1B, LRP2, LRP4* | |
| **Histone methyltransferase/ demethylase activity** GO terms | *EHMT2, KDM4A, KDM4B, KDM4D, KDM5C, KDM6B, KMT2B, KMT2C, NSD1, PHF2, PRDM16, PRDM7, PRDM9, SETD2, SETDB1, SETDB2* | *KDM5A, KMT2D, MECOM* | *JMJD1C, KDM3A, KMT2A, PRMT2* |
| **MAPK signaling pathway** KEGG Pathway | *AKT3, ARRB1, CACNA1A, CACNA1B, CACNA1G, CACNA1I, CACNA1S, CACNA2D1, CACNA2D2, CACNA2D3, CACNB1, CACNB3, CACNG3, CHP2, CHUK, DUSP4, EGF, FGF7, FGF12, FGFR2, FLNC, GRB2, HRAS, HSPA1L, MAP2K3, MAP3K4, MAP3K5, MAP3K11, MAP4K1, MAP4K3, MAP4K4, MAPK10, MAPK8IP3, MOS, MYC, NF1, NTRK1, NTRK2, PAK2, PDGFRA, PDGFRB, PPP3CA, PRKCB, PTPN7, PTPRR, RAF1, RAPGEF2, RASGRF2, RASGRP3, RPS6KA1, RPS6KA5, RPS6KA6, TAOK1, TGFB2* | *BRAF, CACNA1D, CACNA1E, CACNA1H, CACNB2, FGFR3, MECOM, NRAS, PLA2G4A, PTPN5,* ***RASA1****, SOS2,* ***TP53*** | *CACNA1C, IL1B, MAP3K6, MAP3K13, NFATC4, PLA2G4E, PPM1B, RASGRF1, RASGRP1, RPS6KA3, SOS1, STK4* |
| **Protein tyrosine kinase activity** GO terms | *ALK, BLK, CLK2, CLK3, DDR1, DDR2, DSTYK, EGF, EPHA2, EPHA3, EPHA4, EPHA6, EPHB4, EPHB6, ERBB2, ERBB3, FGF7, FGFR2, FLT4, FRK, HSP90AA1, KIT, MAP2K3, MATK, MERTK, NTRK1, NTRK2, PDGFRA, PDGFRB, PKDCC, PTK2B, RET, ROR2, ROS1, SGK223, SRMS, STYK1, SYK, TEC, TTK, TYRO3, ZAP70* | *EPHA5, EPHA7, ERBB4, FGFR3, FLT3, IGF1R, JAK1, KDR, NTRK3, PTK2, TIE1, TTN* | *CDC37, EFNB3, EPHB2, FYN, IL3RA, NRG1, NRP1, STAT5A, TEK, TYK2* |