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Original Research Article

Transcriptome Analysis and Bioinformatics Characterization of Canine Hemangiosarcoma: Potential Therapeutic Targets

Key words

angiosarcoma;
spleen;
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transcriptomic profiling

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Abstract: Canine hemangiosarcoma is an aggressive cancer with a poor prognosis. It originates in the cells that line blood vessels and affects various organs, including the spleen, heart, and liver. Despite its rarity, canine hemangiosarcoma presents significant diagnostic and therapeutic challenges. Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, have a higher susceptibility to Hemangiosarcoma (HSA), suggesting a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. This study aimed to improve our understanding of the molecular mechanisms underlying canine hemangiosarcoma by re-analyzing publicly available RNA sequencing data using bioinformatic techniques in dogs. Our results suggest that the genes *ALB*, *TNNT2*, *VIM* and *CA9* have the potential to be used as novel biomarkers for spleen, heart, and liver HSA in the Golden Retriever breed. Based on our findings, we propose that *STAT3*, *TP53*, *PPARG*, *ATF3*, *CCND1*, and miR-21-5p, miR-92a-3p, and miR-155-5p have the potential to serve as biomarkers for hepatic HSA in Golden Retrievers. In addition, our analysis of splenic HSA datasets from six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA. The identification of these biomarkers enhances our understanding of the molecular mechanisms involved in AS and provides potential targets for therapeutic intervention.

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Introduction

Human Angiosarcoma (AS) is a highly aggressive type of cancer that originates from the cells responsible for blood vessel formation. It is characterized by its aggressive behavior and generally poor prognosis. However, there have been few large-scale genomic studies of the disease in humans due to its rarity, accounting for only 0.01% of all cancers. It is challenging to conduct large clinical trials due to their rarity and genetic heterogeneity to establish treatment guidelines for existing regimens, and the development of novel therapies has also proven to be difficult (1). However, hemangiosarcoma (HSA) is a common type of cancer in dogs that affects endothelial cells, which form blood vessels, and shares many similarities with human AS (2). Therefore, it has been suggested as a relevant model for studying the pathophysiology of the disease. In particular, certain dog breeds, such as golden retrievers, are prone to developing HSA, with up to 20% of cases occurring in this breed, providing a large sample size for studying the disease that would otherwise be inaccessible using only human data. HSA is often aggressive and fatal within a few months of diagnosis, resulting in a need for new diagnostic and therapeutic options for patients (2).

Visceral canine HSA is particularly hazardous, with a high probability of recurrence and a poor prognosis due to the advanced stage of the disease at diagnosis, resulting from multifocal growth and metastases. Histopathologically, human AS and canine HSA are identical and emerge from the same cell of origin. HSA accounts for up to 7% of all malignant tumors in dogs and typically affects the spleen, liver, and the right atrium/auricle of the heart (3, 4). The prognosis for dogs with splenic HSA is extremely unfavorable, with a survival period typically ranging from 10 to 86 days following surgical removal of the tumor without additional treatment. Even when chemotherapy is combined with surgical resection, the survival period extends to only 141 to 179 days (5). Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, are more prone to HSA, indicating that genetic factors may contribute to the development of the disease (6). Visceral HSA commonly metastasizes to the lungs, liver, mesentery, and omentum and can often present concurrently in the spleen and heart. Cutaneous HSA, which occurs in non-pigmented or light-haired skin, is associated with exposure to UV radiation. Compared to cutaneous HSA, visceral HSA has a poorer prognosis due to local infiltration, primary tumor rupture, and/or metastases (7). Overall, the identification of genetic mutations associated with HSA represents a significant step in understanding and treating this disease. Continued research in this area is necessary to fully comprehend the role of genetics in HSA and to develop effective treatment options.

Certain breeds, such as Golden Retrievers, Boxers, and German Shepherds, have a higher susceptibility to HSA, suggesting a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. The aim of this study is to gain a better understanding of the underlying molecular mechanism of canine HSA at the transcriptomic level. In this study, a bioinformatics approach was used to identify differentially expressed genes (DEGs) in tissue samples obtained from the spleen, liver, and heart of Golden Retriever dogs with HSA. Furthermore, DEG analysis was performed to compare different dog breeds with splenic HSA. Our results suggest that the genes *ALB*, *TNNT2*, *VIM* and *CA9* have the potential to be used as novel biomarkers for splenic, cardiac, and liver HSA in the Golden Retriever breed. In addition, our analysis of splenic HSA datasets from six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA.

Material and methods

RNA-Seq Data Sources

This retrospective study was based on cohort data that included 23 samples of canine HSA. The Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) is the most widely used public functional genomics data repository for high-throughput gene expression data. The gene expression profile dataset, BioProject PRJNA562916, and BioSamples SAMN12659339 - SAMN12659361, generated by Megquier et al. (2), were downloaded from the GEO database and used for bioinformatics analysis. The SRR10355666-SRR10355688 dataset contains 23 samples of canine HSA, and it was used in a bioinformatics analysis to identify differentially expressed genes associated with the disease (Table 1). The data was produced using the Illumina HiSeq 2500.

Bioinformatics Analysis

The FastQ files of the obtained sequence reads were analyzed using a local Galaxy installation (8). Initially, the raw reads underwent quality control checks, and then the adapter was trimmed using Trimmomatic (9). All fragmented reads were mapped to the reference genome (CanFam3.1/canFam3) from UCSC (https://genome.ucsc.edu/index.html) using the HISAT2 tool. The alignments were then assembled into full-length transcripts using the StringTie tool (10). Differential expression analysis was performed using DESeq2 (11).

First, we compared RNA-seq data from Golden Retrievers with three types of HSA tissues (spleen, heart, and liver). The following contrasts were used to compare the experimental groups for the Golden Retriever dog breed: splenic HSA versus liver HSA (SPLNvsLVR), splenic HSA versus heart HSA (SPLNvsHRT), and heart HSA versus liver HSA (HRTvsLVR). Second, splenic HSA RNA-Seq data were compared between six dog breeds: Golden Retriever, German Shepherd, Portuguese Water Dog, American Staffordshire Terrier, Parsons Russell Terrier, and Mix (Labrador Retriever). Table 2 lists the groups created to compare the experimental groups and their abbreviations.

Differentially expressed genes were identified using fold change and adjusted p-values (FDR). Genes with FDR <0.05 were assigned as differentially expressed. For up-regulated genes, a log2FC>1 and for downregulated genes, a log2FC<1 was considered statistically significant, along with an FDR <0.05. A volcano plot was constructed using the ggplot2 package in the R language.

Gene Ontology and pathway enrichment analysis of DEGs

We used the KOBAS (12) online analysis database to conduct Gene Ontology (GO) and KEGG pathway enrichment analysis on the DEGs. The enriched terms for GO and pathways for Reactome and KEGG were selected with an adjusted P-value of <0.05.

To confirm the function enrichment analysis, web tools such as DAVID (https://david.ncifcrf.gov/), g:Profiler (https://biit.cs.ut.ee/gprofiler/gost), WebGestalt (http://www.webgestalt.org/), and GeneCodis (https://genecodis.genyo.es/) were used to analyze each list of DEGs. Additionally, GOView (http://www.webgestalt.org/2017/GOView/) was utilized to obtain a more meaningful view and compare GO enrichment results.

PPI Network and Identification of Hub Genes

We performed protein-protein interaction (PPI) network analysis using STRING (13) and Cytoscape (14). The proteins were first mapped to the STRING database, and PPIs with the highest confidence score (≥0.9) were selected. Subsequently, these PPIs were analyzed using Cytoscape. To gain a better understanding of biological systems, it is important to comprehend the modular structures of biological networks since these systems are composed of modules. Seed growth clustering algorithms, of which MCODE is a typical one, can be used to detect functional modules based on the density of protein interaction networks (15). Interactions were visualized and evaluated using Cytoscape, and the hub gene in the functional network was identified. The next step involved applying the Molecular Complex Detection (MCODE) algorithm (16) to identify densely connected protein neighborhoods in the network. To visualize these modules, the MCODE app in Cytoscape was used with specific parameters, including a node score

Table 1: The characteristics of sample information and accession numbers

Accession	Breed	Gender	SRA Study	Tissue
SRR10355666	Golden Retriever	male	SRP227237	Spleen
SRR10355667	Golden Retriever	female	SRP227237	Heart
SRR10355668	Mix (Labrador Retriever)	female	SRP227237	Liver
SRR10355669	Golden Retriever	female	SRP227237	Spleen
SRR10355670	Portuguese Water Dog	female	SRP227237	Spleen
SRR10355671	Golden Retriever	male	SRP227237	Spleen
SRR10355672	Golden Retriever	female	SRP227237	Heart
SRR10355673	American Staffordshire Terrier	female	SRP227237	Spleen
SRR10355674	German Shepherd Dog	male	SRP227237	Spleen
SRR10355675	German Shepherd Dog	male	SRP227237	Spleen
SRR10355676	Golden Retriever	male	SRP227237	Liver
SRR10355677	Golden Retriever	male	SRP227237	Spleen
SRR10355678	Golden Retriever	female	SRP227237	Spleen
SRR10355679	Parsons Russell Terrier	male	SRP227237	Spleen
SRR10355680	Golden Retriever	male	SRP227237	Spleen
SRR10355681	Mix (Labrador Retriever)	male	SRP227237	Spleen
SRR10355682	Mix	female	SRP227237	Spleen
SRR10355683	Golden Retriever	female	SRP227237	Heart
SRR10355684	German Shepherd Dog	male	SRP227237	Spleen
SRR10355685	Portuguese Water Dog	female	SRP227237	Spleen
SRR10355686	Golden Retriever	female	SRP227237	Heart
SRR10355687	Golden Retriever	male	SRP227237	Spleen
SRR10355688	Golden Retriever	female	SRP227237	Spleen

Table 2: The established groups for the purpose of comparing to the splenic hemangiosarcoma between six dog breeds

Comparison groups	Abbreviations	Animal number in each comparison group
Golden Retriever versus German Shepherd Dog	GLDNvsGRMN	11
Golden Retriever versus Portuguese Water Dog	GLDNvsPORTG	10
Golden Retriever versus Parsons Russell Terrier	GLDNvsRUSTRR	9
Golden Retriever versus American Staffordshire Terrier	GLDNvsSTFTRR	9
Golden Retriever versus Mix	GLDNvsMIX	10
Portuguese Water Dog versus Parsons Russell Terrier	PORTGvsRUSTRR	3
Portuguese Water Dog versus American Staffordshire Terrier	PORTGvsSTFTRR	3
German Shepherd Dog versus Portuguese Water Dog	GRMNvsPORTG	5
German Shepherd Dog versus Parsons Russell Terrier	GRMNvsRUSTRR	4
German Shepherd Dog versus American Staffordshire Terrier	GRMNvsSTFTRR	4
Parsons Russell Terrier versus American Staffordshire Terrier	RUSTRRvsSTFTRR	2

Table 3: Differentially expressed genes in splenic hemangiosarcoma among six breeds of dogs

GLDNvsMIX				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
ADAM9	-4,37E+14	4,50E+09	0.009	chr16
SPEG	-2,25E+14	9,55E+09	0.015	chr37
SLPI	3,43E+14	0.0006	0.04	chr24
PORTGvsRUSTRR				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
TYROBP	9,59E+14	1,52E-14	1,40E-12	chr1
PEG10	-1,02E+14	8,53E-13	5,24E-09	chr14
CD40LG	5,95E+14	5,09E+05	1,00E-05	chrX
SLPI	3,03E+14	7,73E+09	0.004	chr24
GOT2	3,03E+14	0.0002	0.009	chr2
KRT222	-2,57E+14	0.0017	0.044	chr9
PORTGvsSTFTRR				
Gene name	**Log2(FC)**	**P-value**	**P-adj**	**Chromosome**
PEG10	-1,11E+14	1,57E-13	5,95E-11	chr14
TYROBP	1,00E+14	1,02E-11	1,75E-08	chr1

SLPI	8,28E+14	9,46E-08	1,37E-04	chr24
NOX5	-8,32E+14	4,50E+00	1,33E-04	chr30
MADCAM1	-7,05E+14	1,05E+04	1,00E-04	chr20
TRIB1	-4,39E+14	8,54E+04	1,19E-04	chr13
GOT2	4,07E+14	1,72E+08	0.00011	chr2
TACR1	-4,50E+14	3,42E+05	0.00019	chr17
SNRPC	3,09E+14	9,42E+09	0.0033	chr12
KRT222	3,64E+14	0.0008	0.02	chr9
RPL26	-2,20E+14	0.002	0.04	chr5

GRMNvsPORTG

Gene name	Log2(FC)	P-value	P-adj	Chromosome
TYROBP	-1,05E+14	1,97E-38	4,00953E-34	chr1
CEACAM23	-3,73E+11	1,13E+08	0.0003	chr1
VIM	1,85E+14	0.0002	0.01	chr2
TRIB1	2,57E+13	0.0009	0.04	chr13

GRMNvsRUSTRR

Gene name	Log2(FC)	P-value	P-adj	Chromosome
GPR83	3,27E+14	2,00E+09	0.004	chr21
KRT222	-3,31E+14	3,15E+09	0.006	chr9
CD40LG	2,99E+13	0.0001	0.01	chrX

GRMNvsSTFTRR

Gene name	Log2(FC)	P-value	P-adj	Chromosome
NOX5	-8,75E+12	2,09E-02	1,35E-07	chr30
MSMO1	-2,96E+14	9,21E+05	1,32E-05	chr15
GPR83	5,54E+14	1,13E+07	1,00E-05	chr21
BRCA2	2,03E+14	3,17E+09	0.001	chr25
XRCC1	1,97E+14	0.0002	0.008	chr1
SNRPC	2,56E+14	0.0007	0.02	chr12
DES	2,47E+14	0.001	0.02	chr37
TACR1	-3,90E+13	0.001	0.03	chr17
SLC37A4	-3,20E+14	0.001	0.03	chr5
MAP2K1	-3,35E+13	0.001	0.03	chr30
CD40	1,75E+14	0.001	0.03	chr24

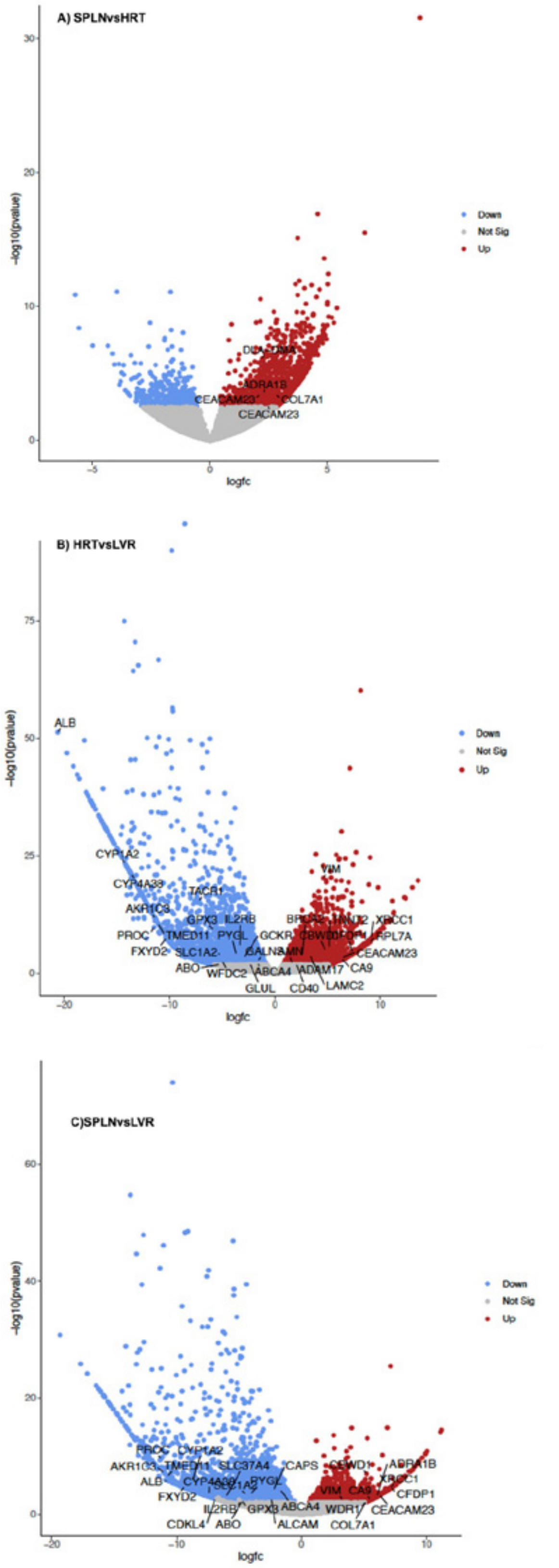


Figure 1: Volcano plots of differentially expressed genes from Golden Retriever dog breed with three hemangiosarcoma tissues. A) SPLNvsHRT, B) HRTvsLVR, and C) SPLNvsLVR, respectively. Red dots represented down-regulated significant genes, and red dots represented up-regulated significant genes. SPLNvsLVR: Splenic Hemangiosarcoma versus Liver Hemangiosarcoma, SPLNvsHRT: Splenic Hemangiosarcoma versus Heart Hemangiosarcoma, and HRTvsLVR: Heart Hemangiosarcoma versus Liver Hemangiosarcoma

cutoff of 0.2, k-core of 2, max depth from the seed of 100, and degree cutoff of 2. Additionally, the Maximal Clique Centrality (MCC) algorithm of cytoHubba (17) was utilized to identify the hub genes.

Transcription factors and miRNAs regulatory network

The miRNet online database (18) was used to identify the transcription factors and microRNAs that target the DEGs. The mirTarbase (19) and DIANA TarBase V.8 (20) tools were used for the prediction of experimentally validated microRNAs. The miRNet database was also used to identify tissue-specific microRNAs. These microRNAs were validated by experiments in the mirTarbase and Tarbase v.8 tools. The 3'UTR sequence of the target gene was obtained from the ENSEMBL website. The miRNA-mRNA targets were validated using the RNA22v2 (21) and RNAhybrid (22) tools.

Results

Identification of DEGs

The spleen is the most common tissue involved in canine HSA, followed by the right atrium/auricle of the heart and the liver. While this type of cancer can affect dogs of any breed, Golden Retrievers have been identified as a breed with an increased genetic predisposition to develop HSA. Gene expression data sets were used to compare altered gene expression in three tissue types of HSA in the Golden Retriever dog breed. The sets used for comparison were SPLNvsLVR, SPLNvsHRT, and HRTvsLVR. A total of four genes were found to be statistically significantly up-regulated in the SPLNvsHRT group. Among the 26 DEGs in the SPLNvsLVR group, 9 genes were up-regulated, and 17 genes were down-regulated. Furthermore, among the 31 DEGs in the HRTvsLVR group, 13 genes were up-regulated, and 18 genes were down-regulated (Figure 1, Supplementary File 1). Interestingly, *CEACAM23* was found to be common in all three groups (Figure 2). A total of five genes, which are CAPS, *SLC37A4*, *CDKL4*, *ALCAM*, and *WDR1*, were only found in the SPLNvsLVR group. On the other hand, the *DLA-DMA* gene was only determined in the SPLNvsHRT group.

Specific breeds like Golden Retrievers, Boxers, and German Shepherds exhibit a higher susceptibility to HSA, indicating a possible genetic basis for disease susceptibility. However, the exact molecular mechanisms underlying the predisposition of these breeds to HSA are not fully understood. In addition, we compared the splenic HSA data from six breeds of dogs to understand the molecular mechanisms underlying this breed predisposition. In the GLDNvsGRMN group, no differentially expressed genes were identified. However, in the GLDNvsPORTG, GLDNvsRUSTRR, and GLDNvsSTFTRR groups, only one gene was found to be differentially expressed in each group: *IL2RB*, *CD40LG*, and *NOX5*, respectively. In the comparison results of the following groups: GLDNvsMIX, PORTGvsRUSTRR, PORTGvsSTFTRR, GRMNvsPORTG, GRMNvsRUSTRR, and GRMNvsSTFTRR we identified 3, 6, 11, 4, 3, and 11 differentially expressed genes as statistically significant, respectively (Table 3, Supplementary File 2).

Functional enrichment analysis of DEGs

The KEGG pathway analysis revealed that the DEGs in the comparison between spleen and liver HSA tissues in the Golden Retriever breed were significantly enriched in Arachidonic acid metabolism and Metabolic pathway. The arachidonic acid pathway is known to have a significant impact on cardiovascular biology and carcinogenesis (23). Moreover, the GO BP enrichment analysis showed that the DEGs group in SPLNvsLVR was significantly enriched in positive regulation of single-strand break repair, positive regulation of DNA ligase activity, and positive regulation of heart rate by epinephrine-norepinephrine. The main enriched GO CC terms were found to be the ERCC4-ERCC1 complex and immunological synapse. The GO MF analysis was significantly enriched in interleukin-15 and interleukin-2 receptor activity. (Supplementary File 3).

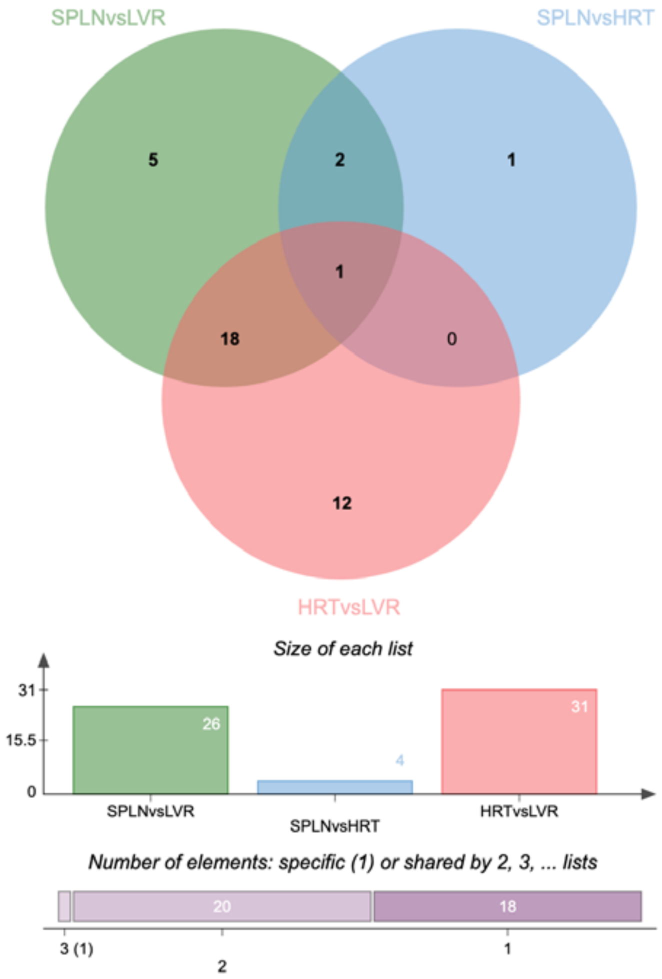


Figure 2: Identification of common differentially expressed genes (DEGs) between SPLNvsHRT, HRTvsLVR, SPLNvsLVR determined from Golden Retriever dog breed. Venn diagram showing 1 DEG (CEACAM23) common to both datasets. SPLNvsHRT: Splenic hemangiosarcoma versus Heart hemangiosarcoma; HRTvsLVR: heart hemangiosarcoma versus liver hemangiosarcoma; SPLNvsLVR: splenic hemangiosarcoma versus liver hemangiosarcoma

The HRTvsLVR group of differentially expressed genes was found to be significantly enriched in several pathways based on KEGG pathway analysis. These pathways included Metabolic pathways and Cardiac muscle contraction. The GO BP enrichment analysis revealed significant enrichment of negative regulation of glucokinase activity and negative regulation of apoptotic process in the HRTvsLVR group, with specific genes implicated in each process. More details can be found in Supplementary File 4.

The genes *DLA-DMA*, *ADRA1B*, *COL7A1*, and *CEACAM23* showed statistically significant differential expression in the comparison between spleen and heart groups, with *DLA-DMA* being uniquely differentially expressed in this group. Meanwhile, *ADRA1B* and *COL7A1* were differentially expressed in both the SPLNvsHRT and spleen and liver comparisons. In the GO BP enrichment analysis, *ADRA1B* was significantly enriched in pathways related to the positive regulation of heart rate by epinephrine-norepinephrine and regulation of cardiac muscle contraction.

According to the results of the comparison of splenic HSA data among six dog breeds, splenic HSA tissues of PORTGvsRUSTRR were significantly enriched in GO BP analysis. *CD40LG* was significantly enriched in pathways related to positive regulation of endothelial cell apoptotic process, and *TYROBP* was significantly enriched in the positive regulation of natural killer cell activation, macrophage activation involved in immune response, and the apoptotic signaling pathway.

In the PORTGvsSTFTRR DEGs group, the GO BP analysis showed significant enrichment in the positive regulation of lymphocyte migration and integrin-mediated signaling pathway. Enrichment analysis indicated that the genes in PORTGvsSTFTRR DEGs were significantly associated with the metabolism of RNA, eukaryotic translation initiation, and mRNA splicing at the Reactome pathway terms. Conversely, the enriched categories for GRMNvsPORTG DEGs were significantly enriched with microRNAs in cancer (cfa05206; *VIM*) in the KEGG pathway terms.

Notably, *MSMO1*, *BRCA2*, *XRCC1*, *DES*, *SLC37A4*, *MAP2K1*, and *CD40* were identified exclusively in the GRMNvsSTFTRR group, whereas *IL2RB* was found only in the GLDNvsPORTG group. In addition, MADCAM1 and *RPL26* genes were unique to the PORTGvsSTFTRR group, and *CEACAM23* and *VIM* genes were identified exclusively in the GRMNvsPORTG group. In addition, *ADAM9* and *SPEG* genes were observed exclusively in the GLDNvsMIX group (Figure 3). These results demonstrate that breed-specific genes are expressed in canine splenic HSA.

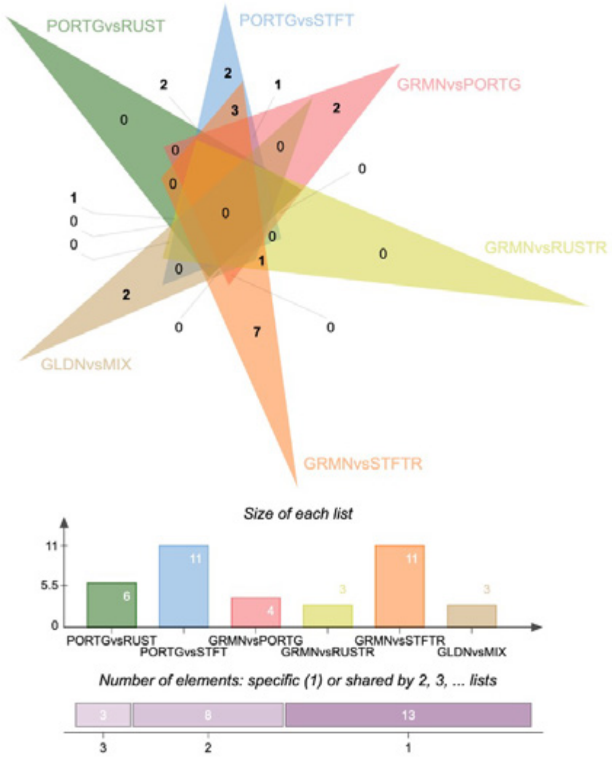


Figure 3: The identification of common and unique differentially expressed genes in splenic hemangiosarcoma among six breeds of dogs. PORTGvsRUSTRR: Portuguese Water Dog versus American Staffordshire Terrier; PORTGvsSTFTRR: Portuguese Water Dog versus American Staffordshire Terrier; GRMNvsPORTG: German Shepherd Dog versus Portuguese Water Dog; GRMNvsRUSTRR: German Shepherd Dog versus Parsons Russell Terrier; GRMNvsSTFTRR: German Shepherd Dog versus American Staffordshire Terrier; GLDNvsMIX: Golden Retriever versus Mix

Identification of PPI Networks and Hub Genes

The comparison between spleen and liver HSA tissues in Golden Retrievers revealed a protein-protein interaction (PPI) network consisting of 8 edges and 11 nodes (Figure 4A). The most highly connected hub genes in this network, as determined by MCC and Cytohubba Degree value, were *ALB*, *CYP1A2*, and *CA9* (Figure 4C). Similarly, in the HRTvsLVR group of Golden Retrievers, the PPI network of DEGs comprised 14 edges and 15 nodes (Figure 4B). The hub genes with the highest connectivity, based on MCC and Cytohubba Degree value, were identified as *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* (Figure 4D). Among the other analyzed groups, no protein-protein interaction network was identified for the differentially expressed genes. These findings provide insights into the key genes and their interactions within the PPI networks associated with the different comparisons, shedding light on the molecular mechanisms underlying spleen and liver HSA in Golden Retrievers.

Prediction of miRNAs and Transcription factors

The miRNet database was utilized to identify the miRNAs and Transcription Factors (TF) targeting the *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* genes (Figure 5). Functional enrichment analysis was also conducted to determine the associated miRNAs and TFs. The results revealed 10 miRNAs targeting *ALB*, 17 miRNAs targeting *CA9*, 18 miRNAs targeting *CYP1A2*, and two miRNAs (hsa-mir-335-5p and hsa-mir-101-3p) targeting *TNNT2*. Additionally, 117 miRNAs were found to target *VIM*. The determined transcription factors for the *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9* genes can be found in Supplementary Table 5. Specifically, 12 transcription factors were identified for *ALB*, 13 for *CA9*, 7 for *CYP1A2*, 12 for *TNNT2*, and 43 for *VIM*. The functional enrichment analysis of miRNAs revealed their involvement in the cell cycle, angiogenesis, and onco-miRNAs. In terms of disease enrichment, miRNAs were significantly enriched in pancreatic neoplasms, malignant neoplasms, cardiovascular diseases, and cardiomyopathy ($p<0.05$). Furthermore, 24 miRNAs were found to be specific to liver tissue, while one miRNA (hsa-let-7b) was specific to heart tissue. (Supplementary Table 6).

Functional enrichment analysis was also conducted for the identified TFs. The disease enrichment analysis revealed that these genes were statistically significantly enriched in HSA and liver neoplasms (Figure 5B,5C). In addition, acute coronary syndrome, heart disease, cardiovascular disease, and liver failure were significantly enriched for *ALB*, *TNNT2*, and *VIM*. (Supplementary Table 7).

Discussion

The One Health or One Medicine interdisciplinary approach aims to enhance human and animal health by examining health concepts at the individual, population, and ecosystem levels. Here, we have demonstrated that by employing dogs as models for human diseases within comparative medicine, particularly focusing on the 400 inherited diseases (2) they share with humans and their spontaneous development of cancer with similar molecular profiles, we can significantly enhance our understanding of the molecular basis of cancer including rare types like AS and HAS and contribute to the development of new treatments.

This research may help us to develop new diagnostic and therapeutic options for AS patients. First, we identified genes with altered expression in splenic, heart, and liver HSA tissues in Golden Retriever dogs. The most highly connected hub genes between the spleen and the liver in HSA tissues of Golden Retrievers were identified as *ALB*, *CYP1A2*, and *CA9*. Similarly, in the HRTvsLVR group of Golden Retrievers, the hub genes were identified as *ALB*, *TNNT2*, *CYP1A2*, *VIM*, and *CA9.*

In our study, we successfully identified the miRNAs and TFs predicted for the *ALB* gene. Conducting a functional enrichment analysis, we discovered that miR-107, miR-155-5p, miR-203-3p, and miR-27a-5p, which are specifically associated with the *ALB* gene, are considered to be oncomiRs. Furthermore, our investigation revealed that miR-1-3p,

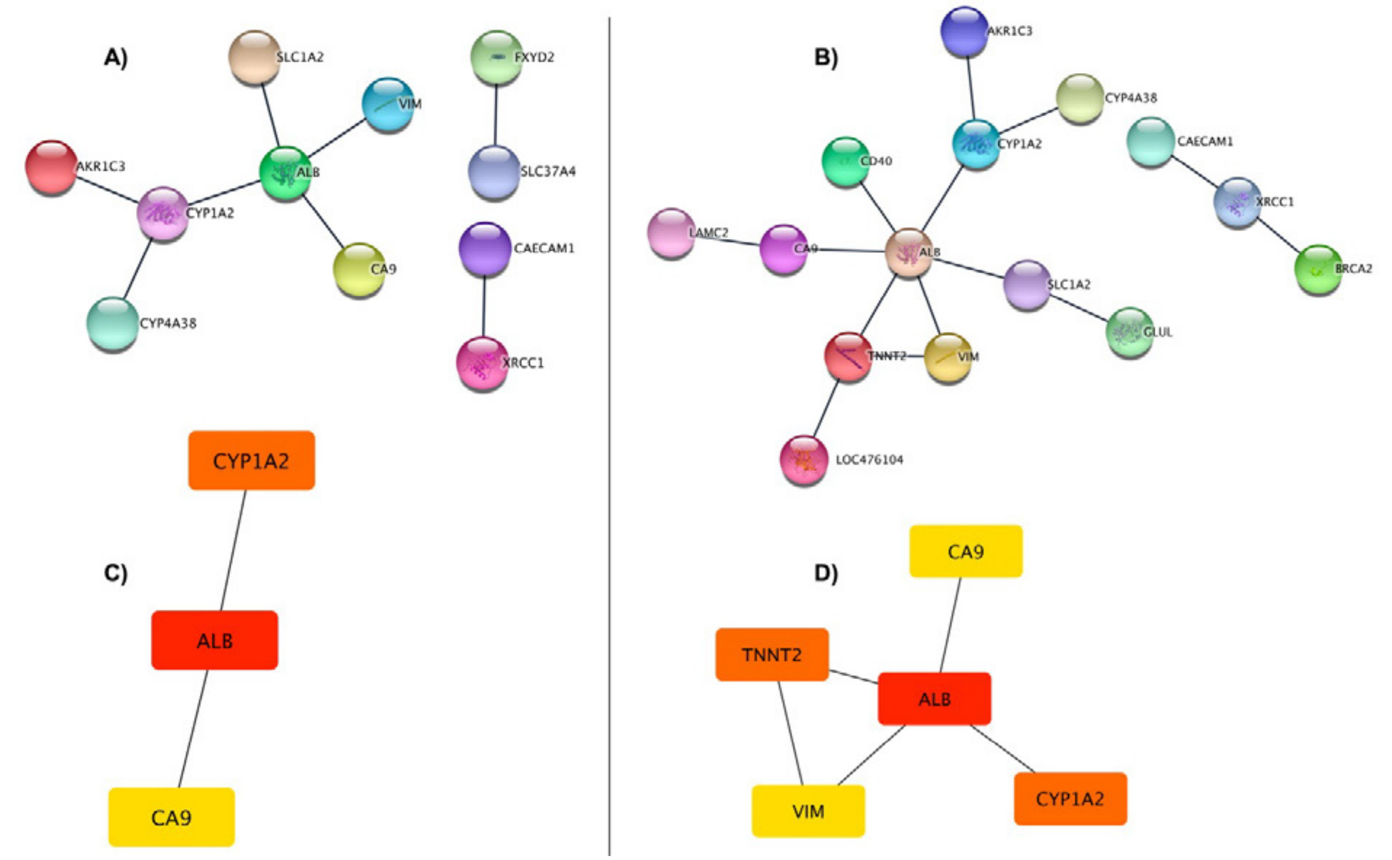
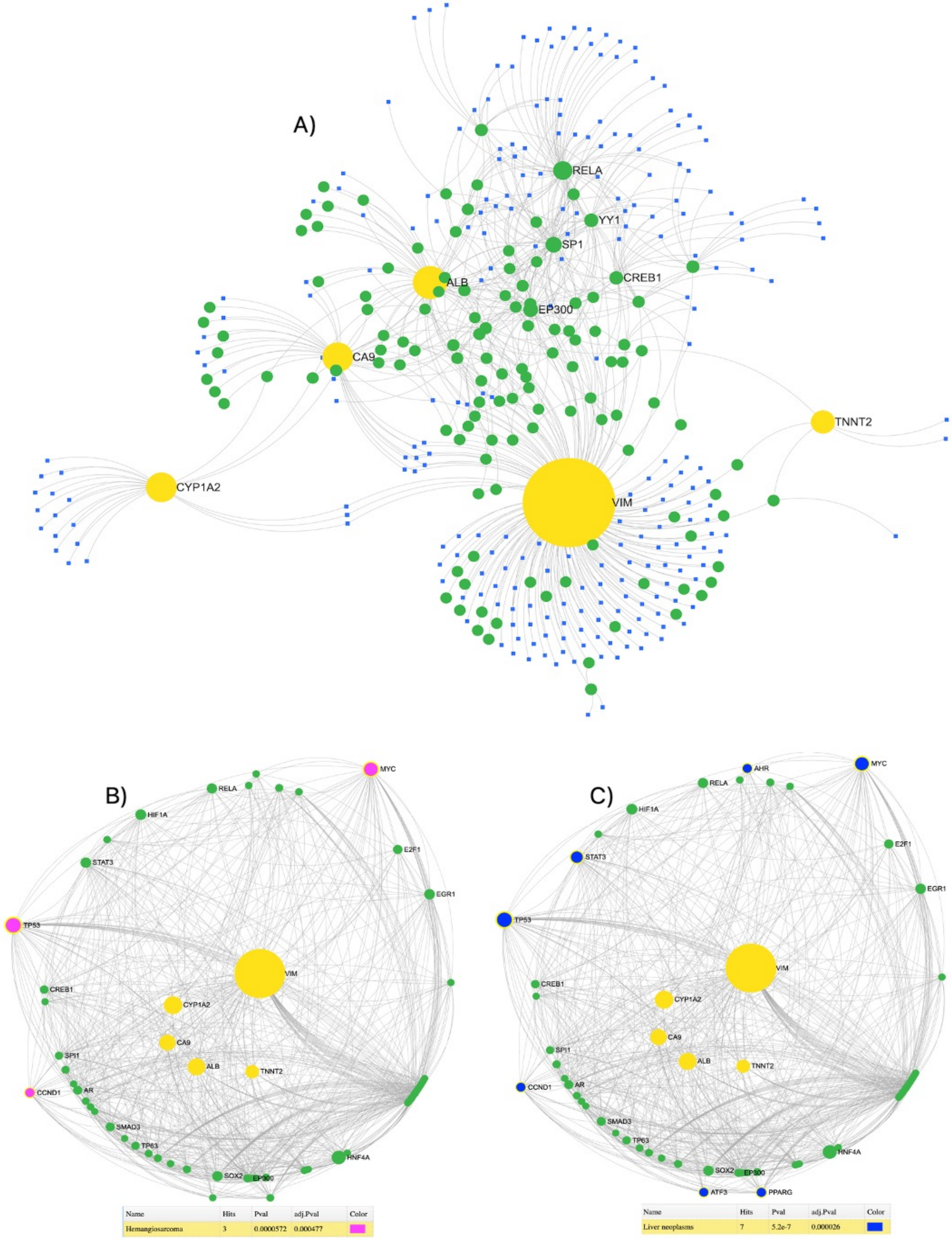


Figure 4: The protein-protein interaction network for the differentially expressed genes in the SPLNvsLVR (A) and HRTvsLVR group (B). Visualization of the networks of the top three hub genes in the SPLNvsLVR (C) and HRTvsLVR group (D). SPLNvsHRT: Splenic hemangiosarcoma versus Heart hemangiosarcoma; HRTvsLVR: heart hemangiosarcoma versus liver hemangiosarcoma; SPLNvsLVR: splenic hemangiosarcoma versus liver hemangiosarcoma

miR-124-3p, and miR-155-5p have specificity to liver tissue. In addition, we performed a functional enrichment analysis for the TFs identified for the *ALB* gene, specifically *STAT3*, which showed statistically significant enrichment in liver neoplasms. *STAT3* has previously been detected in a number of canine malignancies, consistent with our findings. The TFs are proteins responsible for regulating gene expression by binding to specific DNA sequences within promoter regions. They modulate gene expression by pre-transcriptionally activating or repressing downstream genes (25). Our results showed that the *ALB* gene was downregulated in both the SPLNvsLVR and HRTvsLVR groups. Based on this observation, we speculated that *ALB* may play a role in the regulation of liver function during hepatic AS. However, some of the DEGs between HSA in different tissues appear to be tissue-specific genes. This may reflect differences in tissue composition between organs rather than the cancer genes themselves. As a result, we propose that *STAT3*, miR-107, miR-155-5p, miR-203-3p, and miR-27a-5p may serve as potential biomarkers for HSA. Further research is needed to confirm these findings and to investigate the mechanisms by which *ALB* may affect liver HSA progression.

Vimentin (*VIM*), categorized as a type III intermediate filament (IF) protein, holds a prominent position among the IF protein family and has been extensively investigated. Studies have demonstrated the potential role of vimentin (*VIM*) as a significant biomarker in specific histological variants of canine liver cancer, such as moderately differentiated hepatocellular carcinoma, mixed carcinoma, and poorly differentiated carcinoma (26). The research conducted by Sawa et al. (27) provided support for the diagnostic sensitivity of rapid immunocytochemistry (ICC) in detecting vimentin in the neoplastic tissues of dogs. Further support for the significance of vimentin in canine neoplastic conditions was provided by the observations made by Shiga and Shirota (28). Their study revealed that vimentin exhibited co-expression with bile duct-type cytokeratin in well-differentiated hepatocellular carcinoma in a dog. These findings highlighted the role of vimentin as an important marker for determining the cellular origin of tumors in the liver. In accordance with the above study, our results show that *VIM* may be important in liver function and cancer progression. In our study, we conducted an analysis and identified a total of 117 miRNAs that are predicted to target the *VIM* gene and also 43 transcription factors for *VIM*. Among these miRNAs, several oncomiRs were identified.

These miRNAs have been associated with oncogenic properties and are implicated in various cancer-related processes. Furthermore, our analysis revealed that miR-21-5p, miR-92a-3p, and miR-155-5p exhibit both liver tissue



Name	Hits	Pval	adj.Pval	Color
Hemangiosarcoma	3	0.0000572	0.000477	🟪

Name	Hits	Pval	adj.Pval	Color
Liver neoplasms	7	5.2e-7	0.000026	🟦

Figure 5. Target gene-miRNA-transcription factors (TF) interaction network between *ALB, TNNT2, CYP1A2, VIM*, and *CA9* genes, the TF, and miRNAs. Green nodes represent TFs, yellow nodes represent genes, and blue nodes represent miRNAs (A). Visualization of the functional enrichment results. The disease enrichment analysis revealed that these genes were statistically significantly enriched in (B) hemangiosarcoma (*MYC, TP53, CCND1*) and (C) liver neoplasms (*AHR, STAT3, MYC, TP53, PPARG, ATF3, CCND1*)

specificity and oncomiR characteristics. This indicates that these miRNAs may play a dual role in liver tissue and contribute to the development and progression of liver-related cancers.

Based on our findings, we propose that *STAT3*, *TP53*, *PPARG*, *ATF3*, *CCND1*, and miR-21-5p, miR-92a-3p, and miR-155-5p have the potential to serve as biomarkers for hepatics HSA in Golden Retrievers. These molecules have demonstrated significant associations with the *VIM* gene and show relevance to liver neoplasms. By further investigating their expression patterns and functional roles, these biomarkers may contribute to the early detection, diagnosis, and prognosis of HSA in golden retrievers. However, additional research is needed to validate and establish the clinical utility of these biomarkers in the context of HSA.

Yoshikawa et al. (5) discovered that miR-214 5AE is a promising novel chemotherapeutic agent for the treatment of canine HSA. In a study by Grimes et al. (29) comparing healthy dogs with a splenic mass, the researchers identified five specific miRNAs (miR-214-3p, miR-452, miR-494-3p, miR-497-5p, and miR-543) in the circulation. These five miRNAs were found to be up-regulated in dogs with HSA or hematoma compared to healthy control dogs. In this study, several transcription factors, including *CUX1*, *EGR1*, *HIF1A*, *SRF*, and *STAT3*, were predicted to be targeted by miR-214. Specifically, *CUX1* was found to regulate the *VIM* gene, *EGR1* was associated with the *ALB* and *TNNT2* genes, *STAT3* was linked to the *ALB*, *TNNT2*, and *VIM* genes, *HIF1A* was implicated in the regulation of the *CA9* gene, and *SRF* was found to modulate the *TNNT2* gene. Both miRNAs and TFs can work together, exerting their regulatory functions on shared target genes. Moreover, they can also influence each other's expression (25). Therefore, based on our findings and consistent with previous studies, we propose that miR-214 has the potential to serve as a biomarker for HSA.

In conclusion, our study suggests that the genes *ALB*, *TNNT2*, *VIM*, and *CA9* have the potential to be used as novel biomarkers for splenic, cardiac, and liver HSA in the golden retriever dog breed. Additionally, our analysis of splenic HSA datasets across six different dog breeds reveals the expression of breed-specific genes in canine splenic HSA. Our findings have the potential to contribute to the development of novel diagnostic and therapeutic approaches for patients with AS. However, it is important to validate these findings with additional clinical case data. The identification of these biomarkers enhances our understanding of the molecular mechanisms involved in AS and provides potential targets for therapeutic interventions.

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Declarations

Ethical Approval: not applicable. Consent to Participate: all authors contributed to this project.

Consent for Publication: all authors are agreed.

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Author contributions: Özge Özmen contributed to conceptualization, methodology design, data collection, visualization of data, selection, or development of software tools, writing the original draft, validation of results, acquiring resources, supervising the study's progress, and obtaining funding. Berna Kaya was involved in developing the theoretical framework and manuscript preparation. Kardelen Karaman contributed to developing the theoretical framework, drafting the manuscript, and arranging the figures. All authors participated in discussions on the results and provided comments on the manuscript.

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Analiza transkriptoma in bioinformacijska karakterizacija hemangiosarkoma pri psih: potencialne terapevtske tarče

Ö. Özmen, B. Kaya, K. Karaman

Izvleček: Pasji hemangiosarkom (HSA) je agresiven rak s slabo prognozo. Nastane v celicah, ki obdajajo krvne žile, in prizadene različne organe, vključno z vranico, srcem in jetri. Kljub redki pojavnosti predstavlja velike diagnostične in terapevtske izzive. Nekatere pasme, kot so zlati prinašalci, bokserji in nemški ovčarji, so dovzetnejše za hemangiosarkom, kar kaže na možno genetsko podlago dovzetnosti za bolezen. Vendar pa natančni molekularni mehanizmi, ki določajo nagnjenost teh pasem k HSA, še niso povsem pojasnjeni. Namen te študije je bil izboljšati naše razumevanje molekularnih mehanizmov za določanje hemangiosarkoma pri psih, in sicer s ponovno analizo javno dostopnih podatkov o sekvenciranju RNA z uporabo bioinformacijskih tehnik pri psih. Naši rezultati kažejo, da bi se geni ALB, TNNT2, VIM in CA9 lahko uporabili kot novi biomarkerji za HSA vranice, srca in jeter pri pasmi zlati prinašalec. Na podlagi naših ugotovitev predlagamo, da bi STAT3, TP53, PPARG, ATF3, CCND1 ter miR-21-5p, miR-92a-3p in miR-155-5p služili kot biomarkerji za jetrni HSA pri zlatih prinašalcih. Poleg tega naša analiza nabora podatkov HSA vranice šestih različnih pasem psov razkriva izražanje pasemsko značilnih genov v HSA vranice psov. Identifikacija teh biomarkerjev krepi naše razumevanje molekularnih mehanizmov angiosarkoma (AS) in predlaga potencialne tarče za zdravljenje.

Ključne besede: angiosarkom; vranica; srce; jetra; primerjalna onkologija; transkriptomsko profiliranje