Reviews

Current understanding of comparative pathology and prospective research approaches for canine hemangiosarcoma

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Running title: Comparative pathology and research approaches for HSA

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Abstract

Hemangiosarcoma (HSA) is a malignant tumor originating from endothelial cells. HSA typically develops in dogs, but is rare in other animals, including humans. Although surgery and chemotherapy are conventional treatments for HSA, neither treatment can significantly improve patient prognosis. To develop novel and effective therapeutics, a deeper understanding of HSA pathogenesis must be acquired. However, the limited research tools for HSA have been unable to make a breakthrough; therefore, it is crucial to widely utilize or establish novel research tools such as patient-derived xenograft models, organoids, and chicken embryo xenograft models. The pathogenesis of the human counterpart of HSA, angiosarcoma (AS), also remains incompletely understood, preventing the extrapolation of findings from humans to dogs, unlike other diseases. In this review, we summarize the clinicopathological and morphological features of HSA, and then we discuss the current understanding of the molecular pathology of HSA. Finally, we highlight promising research tools that may accelerate HSA basic research toward developing novel therapeutics. We also briefly summarize AS to help researchers comprehend HSA from the perspective of comparative pathology.

Keywords: angiosarcoma, basic research, hemangiosarcoma, molecular pathology, PDX models

1. Introduction

Hemangiosarcoma (HSA) is a malignant tumor derived from endothelial cells. Dogs are the most susceptible species to this disease, showing high frequency and mortality. Over the past few decades, veterinary oncology has seen significant advancements in the development of novel treatments for tumors including molecular target drugs. Despite the progress, there remains a lack of effective treatments for HSA. Currently, surgery and chemotherapy are common treatment methods; however, they are limited in their ability to significantly improve patient prognosis. One reason for this is the incomplete understanding of the molecular pathogenesis of HSA, which prevents researchers from identifying effective therapeutic targets. Another factor is the lack of insight into the pathogenesis of angiosarcoma (AS), the human counterpart of HSA, which renders the extrapolation of knowledge from humans to dogs unfeasible. In this review, we first present a comprehensive summary of the characteristics of HSA and then delve into its molecular pathology and the current research landscape, highlighting promising research tools for developing effective treatments. Additionally, we discuss AS and describe its similarities and differences with HSA. We believe that comparative analyses between HSA and AS could advance research on both diseases.

2. Clinicopathology

HSA occurs in various domestic animals, including dogs, cats, horses, ferrets, and rabbits. Dogs are the most susceptible species, and HSA accounts for 1.3-2.8% of all canine tumors (Aupperle-Lellbach et al., 2022; Baioni et al., 2017; Dobson et al., 2002; Grüntzig et al., 2016). The spleen is the most frequent primary location, and splenic HSA is prone to containing large amounts of blood and rupturing due to its fragility, leading to hemoabdomen and sudden death (Prymak et al., 1988). Other commonly affected organs include the skin, liver, kidneys, right atrium of the heart, and mesentery, but HSA also occurs at low frequencies in skeletal muscles, bones, eyes, and oral cavities (Fig. 1) (Brown et al., 1985; Finotello et al., 2017; Sorenmo et al., 2000, 2004). HSA is highly invasive and often metastasizes to distant organs such as the liver, lung, omentum, and diaphragm through blood vessels or peritoneal dissemination (Batschinski et al., 2018; Story et al., 2020; Wendelburg et al., 2015). It is common for metastasis to be recognized even at the time of identification of the primary lesion upon presentation.

Although the cause of the high frequency of HSA in dogs remains elusive, breeds have been identified as potential contributors to its development. Some breeds, namely Golden Retrievers, German Shepherds, and Boxers, are reported to have a disproportionately high risk of HSA development (Batschinski et al., 2018; Carnio et al., 2020; Grüntzig et al., 2016; Robinson et al.,

2020; Schultheiss, 2004). One study noted that Golden Retrievers exhibit distinct gene expression profiles compared to other breeds. The key to endothelial tumorigenesis might be hidden in this signature (Tamburini et al., 2009).

AS has different characteristics from HSA in terms of frequency, primary affected organs, and prognosis (Fig. 1) (Colas et al., 2020; Schöffski et al., 2021; Smrke et al., 2020; Wang et al., 2017). AS is a rare tumor, affecting approximately one in a million individuals per year (Colas et al., 2020; Friedrich et al., 2021). The most common primary site is the skin, particularly in the head and neck region. Breast AS is unique to women and occurs as a secondary lesion after radiation therapy for breast cancer. Unlike canine HSA, the visceral form of AS develops less frequently and shows more favorable outcomes when compared to cutaneous AS. The overall 5-year survival rate of visceral AS without treatment is 37%, while that of cutaneous AS is 28% (Smrke et al., 2020; Wang et al., 2017). Sun exposure, radiation therapy, and lymphedema have been reported as extrinsic risk factors for AS development (Colas et al., 2020).

3. Gross pathology, histopathology, and immunohistochemical characteristics

Grossly, HSA is often observed as single or multiple nodules or masses of varying sizes (Fig. 2). HSA has a dark red color in most cases due to substantial blood accumulation within the tumor tissue, but there are some cases that appear as solid white-tan tumor masses without blood accumulation (Valli et al., 2017). Diagnosing HSA based solely on gross appearance is challenging because it can be mistaken for other mass lesions such as hematoma, hemangioma, or other sarcomas. In cases of dark-red splenic masses, the presence of metastases is the most reliable evidence to diagnose HSA (Valli et al., 2017).

Microscopically, the presence of undifferentiated endothelial cells is the typical morphological feature of HSA. However, their morphologies are inconsistent, with each case exhibiting varying mitotic rates, proliferation patterns, and degrees of nuclear and cellular atypia. Despite several proposed morphological classifications for HSA, no growth pattern has been demonstrated to have a significant association with prognosis (Gamlem and Nordstoga, 2008; Göritz et al., 2013; Kim et al., 2015; Otsuka et al., 2022; Roccabianca et al., 2020; Rozolen et al., 2021; Valli et al., 2017). The updated classification of HSA divides HSA into three subtypes: conventional/well-differentiated, Kaposiform (spindle cell), and epithelioid (Roccabianca et al., 2020). It is important to note that despite the aggressive behavior of HSA, the grading system based on microscopic characteristics and morphologies is not useful for predicting patient prognoses although mitotic rates have been reported to have a negative correlation with survival time (Göritz et al., 2013; Moore et al., 2017; Ogilvie et al., 1996).

In cases where it is challenging to distinguish HSA from morphologically similar tumors, immunohistochemistry is valuable for definitive diagnosis. To distinguish between well-

differentiated HSA and hemangioma, VEGFR3 and Ki-67 are useful markers as they show higher expression in HSA than in hemangioma (Sabattini and Bettini, 2009). For epithelioid HSA, which is often confused with other malignant tumors such as undifferentiated carcinoma and metastatic amelanotic melanoma, endothelial markers such as CD31 and von Willebrand Factor (vWF) are helpful for differentiation. However, it has been noted that these markers do not express in some HSA cases (Fig. 3) (García-Iglesias et al., 2020; Göritz et al., 2013; Maharani et al., 2018; Roccabianca et al., 2020; Sabattini and Bettini, 2009), requiring the identification of alternative diagnostic markers especially for epithelioid HSA.

In humans, the definition of AS encompasses tumors derived from not only blood vessels but also lymphatic vessels. Grossly, most early-stage AS cases resemble hematoma but end up forming nodules, plaques, or papules (Ronchi et al., 2020). Histologically, AS exhibits similar patterns to the three morphological subtypes of canine HSA described above (Fig. 4) (Gamlem and Nordstoga, 2008; Neuhauser et al., 2000), although it remains uncertain if the biological behaviors of HSA and AS are comparable. Similar to HSA, the distinction between epithelioid AS and other malignant tumors is also challenging. The expressions of CD31 and vWF have been conventionally used to differentiate AS from other tumors, yet in some cases these markers do not clearly express. FLI-1, a transcriptional factor involved in endothelial cell differentiation, has been shown to be a more sensitive marker for epithelioid AS (Wu et al., 2015).

4. Molecular pathology

Researchers have been investigating the molecular mechanisms underlying HSA pathogenesis to identify effective therapeutic targets and improve patient outcomes. In this section, we will focus on recent discoveries in the molecular pathology of HSA, including the examination of somatic gene mutations, epigenetic factors, and cancer stem/progenitor cells.

4.1. Somatic gene mutations

Somatic gene mutations play a key role in the initiation of tumors by activating oncogenic pathways and/or disrupting tumor suppressor functions. In HSA, the most frequently reported mutated genes are *TP53* and *PIK3CA*, with genes such as *PTEN*, *PLCG1*, and *PIK3R1* less frequently mutated (Megquier et al., 2019; Wang et al., 2020; Wong et al., 2021, 2022). These mutations are involved in the DNA repair system or common oncogenic pathways such as the PI3K/AKT/mTOR and MAPK/ERK signaling pathways. *In vitro* studies have demonstrated that the mTORC2/AKT/4E-BP1 pathway is constitutively activated in HSA cell lines and that PI3K inhibition can induce apoptosis and also reduce proliferation and migration ability (Kavoussi, 2020; Maeda et al., 2022; Murai et al., 2012). A clinical trial in dogs with HSA, however, found that administration of toceranib phosphate, a multi-kinase inhibitor that targets the PI3K/AKT/mTOR

and MAPK/ERK pathways, did not improve patient survival times when it was used in combination with doxorubicin treatment (Gardner et al., 2015; London et al., 2003).

A comparative genetic mutation analysis revealed that cutaneous HSA exhibited a UV mutational signature similar to cutaneous AS, suggesting that genetic mutations caused by sun exposure are highly associated with the development in both canine HSA and human AS in the skin (Wong et al., 2021). Additionally, the presence of heterogeneous mutations of both driver and passenger genes within intra- and inter-tumor tissues in the same patient suggests that polyclonal tumorigenesis contributes to HSA development during tumor evolution (Wong et al., 2022).

In AS, in addition to the commonly mutated genes shared with HSA such as *PIK3CA* and *TP53*, some genes are uniquely mutated (Wang et al., 2020; Wong et al., 2021). These genes, however, are involved in the same oncogenic pathways found in HSA, suggesting that convergent effects contribute to AS and HSA tumorigenesis similar to the development of osteosarcoma in both species (LeBlanc and Mazcko, 2020). One study indicated that combined inhibition of MEK and mTOR resulted in synergistic suppression of AS cell proliferation (Andersen et al., 2015). Another somatic mutation uniquely to AS is the fusion gene NUP160-SLC43A3 (Shimozono et al., 2015). The pathways or cellular events affected by NUP160 and/or SLC43A3 may play a crucial role in the development of AS since the fusion gene allowed fibroblasts which formed tumors that resemble AS (Shimozono et al., 2015).

4.2. Epigenetic factors

Epigenetics is crucial for the pathogenesis of various diseases including tumors. It regulates a wide range of gene expressions and can affect multiple pathways. Epigenetic status can be switched to either transcription active or repressive state depending on the context of the cells. Epigenetic landscapes in tumor cells are different from normal cells and function as a mediator of plasticity in tumors (Feinberg and Levchenko, 2023). Identifying the HSA-specific epigenetic landscape and rewriting it to deprive the malignant phenotype can lead to the development of novel therapeutics for HSA.

Alterations in DNA methylation status have been widely recognized as a hallmark of cancer malignancy (Skvortsova et al., 2019). Increased DNA methylation rates in promoter regions result in gene suppression, while global reductions in DNA methylation can lead to genome instability (Skvortsova et al., 2019). Global DNA methylation status in HSA has yet to be widely explored, but one report has indicated that the DNA methylation profile of LINE-1 in HSA patients was significantly lower than that of other malignant tumors and benign lesions (Sato et al., 2023).

The role of histone modifications in the pathogenesis of HSA also remains unclear, but our recent findings indicate that the expression level of KDM2B is significantly elevated in HSA cell

lines and clinical samples compared to normal endothelial cells, and that KDM2B expression levels were associated with HSA disease progression (Gulay et al., 2022, 2021). Silencing of KDM2B resulted in HSA cell death by impairing DNA damage responses *in vitro* and decreased tumor volume *in vivo* (Gulay et al., 2021). Treatment with GSK-J4, a histone demethylase inhibitor, suppressed HSA tumor growth but was unable to show tumor regression *in vivo* (Gulay et al., 2021). Given that GSK-J4 can inhibit multiple types of histone demethylase, a KDM2B-specific inhibitor could be more effective although it has not yet become available. Additionally, our data indicated that histone acetylation levels are higher in HSA cell lines compared to normal endothelial cells, and it presented heterogeneous histone acetylation patterns in HSA tumor cells of clinical samples (Suzuki et al., 2022). Treatment with JQ1, a small molecule inhibitor for proteins recognizing acetylated histones, induced HSA cell death via apoptosis *in vitro* and significantly suppressed the growth of canine and murine HSA cells *in vivo* (Suzuki et al., 2022).

miRNA expression profiles in splenic HSA differ from those in normal spleens and splenic nodular hyperplasia (Grimes et al., 2016). Intracellular miRNA-214 expression was downregulated in HSA, and its administration inhibited HSA cell proliferation *in vitro* and intraperitoneal dissemination *in vivo* via induction of apoptosis (Yoshikawa et al., 2022, 2020). Moreover, HSA and AS cell lines overexpressed miRNA-214 in extracellular spaces, and its concentration in the plasma of dogs with HSA has been reported to be higher compared to dogs with normal spleens and splenomegaly (Heishima et al., 2015). These findings suggest that miRNA-214 could potentially serve as a diagnostic marker for HSA.

In humans, genome-wide analysis of AS has revealed that DNA methylation patterns vary depending on clinical subtypes, overall survival, and chromosomal instability (Weidema et al., 2020). The global level of trimethylated histone H3 at lysine 27, which is known to be a transcriptional repression marker, was decreased in radiation-associated AS compared to normal endothelial cells (Mentzel and Kiss, 2018; Panse et al., 2021). The expression of a histone deacetylase SIRT1 is higher in AS than in hemangioma and its suppression resulted in a decrease in cell growth and migration in AS cell lines (Chosokabe et al., 2019). Downregulation of miRNA-340 and miRNA-497-5p in AS cell lines has been reported, and their overexpression was able to inhibit cell proliferation and invasion (Chen et al., 2016; Wang and Song, 2018). miRNA-17-92 clusters are upregulated in MYC-amplified AS, leading to the downregulation of the anti-angiogenic protein thrombospondin-1 (Italiano et al., 2012). Although it is still unclear whether HSA and AS have similar epigenetic landscapes, comparative epigenetic analysis could provide more insights into both diseases.

4.3. Cancer Stem/Progenitor Cells

Cancer stem cells (CSCs) are a subpopulation of tumor cells characterized by self-renewal

and differentiation capabilities. They have been associated with malignant phenotypes such as tumor progression, drug resistance, metastasis, and recurrence. Therefore, gaining a deeper understanding of CSCs is important for the development of novel therapeutic strategies. The origin of HSA has yet to be identified, but several mechanisms have been postulated. Some studies suggest that hemangioblasts could be the origin of HSA tumor cells, while others indicated that hematopoietic progenitor cell (HPC) markers (CD34, CD117, and CD133) are expressed in HSA cells albeit their expression levels varied depending on studies (Kakiuchi-Kiyota et al., 2020; Lamerato-Kozicki et al., 2006). Despite researchers' efforts, unfortunately, no specific CSC markers for HSA have been identified. We previously tried to enrich CSCs by culturing HSA cell lines in a low-nutrient condition with cytokine supplementation. The results indicated that NOTCH2, a member of the NOTCH signaling receptors, was highly expressed in the proliferating cells in this culture condition (Aoshima et al., 2018). Given that HSA tumor cells in clinical cases also expressed NOTCH2 higher than normal endothelial cells, NOTCH2 signaling could play an important role in HSA pathogenesis (Aoshima et al., 2018). Another research group used sphere culture in a serum-free condition to enrich CSCs from HSA cell lines and found increased positivity for HPC markers and characteristics of elevated phagocytosis, adipogenesis, and drug resistance (Gorden et al., 2014; Khammanivong et al., 2016; Kim et al., 2018).

CSC markers of AS remain unclear, despite the identification of typical CSC markers in various other human cancers. A report has suggested that the expressions of HPC markers were higher in AS than in hemangioma; however, no studies have been conducted to isolate and characterize CSCs in AS cell lines or tumor tissues using these markers (Liu et al., 2013).

5. Research tools and techniques for HSA

In the sections above, we delved into the pathological aspects of HSA and highlighted the gaps in knowledge about molecular pathogenesis. This section provides a synopsis of the currently available tools for HSA research and proposes promising tools and resources that could advance basic studies on HSA.

The majority of basic research has utilized cell lines and clinical samples obtained from dog patients (Fosmire et al., 2004; Kodama et al., 2009; Murai et al., 2012; Thamm et al., 2006). Cell lines are favored for their ease of handling and low maintenance costs in the laboratory. Gene and protein expressions can be manipulated through genetic engineering, facilitating investigations of their functions. Chemical and drug treatments can also be easily performed using cell lines. However, these tools have some limitations, one of which is the inability to replicate the characteristics of original tumors in patients. During the process to establish cell lines from tumor tissues, only cells that can survive under *in vitro* culture conditions are selected. They adapt to the conditions basically determined by nutrition-rich medium, plastic plates, and 5% CO_2, which are

very different from *in vivo* conditions. Another example is that cell lines lack non-neoplastic components in tumors such as fibroblasts, immune cells, blood vessels, and the extracellular matrix, all of which play crucial roles in supporting tumor cell survival, proliferation, and immune evasion through the secretion of chemicals or direct communication with tumor cells (Hanahan, 2022). Long-term culture of tumor cell lines without the support of these components results in a loss of their original characteristics through adaptation to *in vitro* conditions. These limitations resulted in inconsistent reports of drug responses in experiments using cell line-derived xenograft models and clinical trials (Johnson et al., 2001), which highlights the need for alternative research tools to improve therapeutic predictions and treatments for HSA. Clinical samples such as fresh, frozen, and formalin-fixed paraffin-embedded tumor tissues have also been used for basic research. These samples allow for a direct investigation of the tumor cell characteristics in patients, facilitating the translation of laboratory findings to a clinical setting. However, it is challenging to obtain a sufficient number of samples for research, and individual differences such as breed, location, and malignancy must be taken into consideration when comparing and interpreting the results. To date, HSA research has primarily relied on cell lines and clinical samples; therefore, alternative research tools have been proposed to advance basic research on HSA.

One promising research tool is patient-derived xenograft (PDX) models. These models are established by transplanting patient tumor tissues directly into immunodeficient mice and passaging at least three times in different individuals, enabling tumor cells to survive in *in vivo* environments with continuous support from non-neoplastic components. Given that PDX models do not need culturing *in vitro*, they can retain the original characteristics of tumors and have shown high potential for clinical applications in colorectal cancer, squamous cell carcinoma, and renal cell carcinoma in humans (Bertotti et al., 2011; Keysar et al., 2013; Migliardi et al., 2012; Sivanand et al., 2012). PDX models for HSA have been established in nude mice, and PDX-derived HSA cell lines have been isolated (Kodama et al., 2009; Murai et al., 2012). We have also established HSA PDX models from two dog HSA patients using nude mice and verified that they exhibit similar histopathological features to the original patient tumors (Fig. 5). However, to ensure the quality as research tools that reflect characteristics of original tumors, it is necessary to evaluate that the PDX models maintain the morphologies, proliferative activities, gene mutations, and transcriptional profiles. In human medicine, a substantial number of PDX models have been established and validated through various methods such as histopathological analysis, single nucleotide polymorphism (SNP) analysis, and short tandem repeat (STR) analysis (Meehan et al., 2017; Woo et al., 2019). However, few reports of PDX model establishment have been published in veterinary medicine, and there is still no standard guideline for validation. One study evaluated the characteristics of feline mammary gland tumor PDX models through histological, immunohistochemical, and STR analyses (Chuang et al., 2021). To ensure the quality and reliability

of PDX models in veterinary medicine, it is imperative to establish guidelines for their establishment and evaluation.

Several other research methods and tools have been attempted to better understand HSA pathogenesis. Sphere assay is an important method to study undifferentiated tumor cells including CSCs, and one research team was able to generate HSA tumor spheres from HSA cell lines, and successfully evaluated their differentiation ability (Gorden et al., 2014). We previously investigated the undifferentiated status of HSA cell lines using a different method by which HSA cell lines were cultured under serum-free conditions in an adherent manner (Aoshima et al., 2018). Given that several types of undifferentiated cells exist and could be isolated in different culture conditions, multiple methods should be applied to investigate the characteristics of undifferentiated HSA cells. Organoids are three-dimensional models that recapitulate the 3D structures and components of original tumors in a culture dish. HSA organoids have not yet been established, but they would allow researchers to analyze the responses of HSA to candidate therapeutic drugs and to predict their effects more accurately *in vitro* than using cell lines. Chick embryo tumor xenograft models are another valuable tool for evaluating the therapeutic potential of drugs and the metastatic capacity of tumors. This model can be established by transplanting tumor cells from patients onto the chorioallantoic membrane (Klingenberg et al., 2014; Pawlikowska et al., 2020; Sys et al., 2012; Vu et al., 2018; Xiao et al., 2015). Due to the nutrient-rich condition and incomplete immune system of the membrane, tumor formation, vascularization, and even metastases are developed within a week. Although the host immune system is in the process of development, the main components of the immune system such as macrophages, lymphocytes, and heterophils are present (Vu et al., 2018). The chick embryo model of HSA has not yet been reported, but it is worth establishing to analyze HSA pathogenesis and drug responses faster than using mouse models.

Whole genome/transcriptome sequencing has become increasingly common in veterinary medicine. In the HSA study, a growing number of papers have been showing data with these techniques, revealing novel molecular pathogenesis of HSA and similar genetic mutations between HSA and AS (Megquier et al., 2019; Mukai et al., 2020; Tamburini et al., 2010; Wong et al., 2021). However, it is important to note that these analyses were done using data obtained from bulk tumor tissues. These data include not only tumor cells but also non-tumor cells such as immune cells and fibroblasts. Necrotic tissues and erythrocytes can increase the noise in the results. To investigate detailed characteristics and tumor heterogeneity in HSA, single-cell sequencing is a promising option. Spatial gene expression analysis is another method to unveil the characteristics of HSA tumor cells based on their location in tissues. Given that neither single-cell analysis nor spatial gene expression analysis has been reported in HSA and AS, these experiments should provide useful information for their basic research.

In summary, current limitations in the available research tools for the study of HSA

highlight the use or need for the development of new research tools such as PDX models and organoids. These tools can provide a deeper understanding of HSA pathogenesis by taking into consideration factors such as tumor microenvironment and heterogeneity, with the ultimate goal of advancing clinical applications and improving therapeutic outcomes.

6. Conclusion

The molecular pathogenesis of HSA remains poorly understood. Novel research tools for HSA such as PDX models, organoids, and chick embryo models could help overcome current limitations in basic research for HSA. Additionally, using models with advanced techniques such as single-cell analysis and spatial gene expression analysis could provide results that more accurately reflect HSA pathogenesis in patients. In the veterinary field, it is a common approach to investigate animal diseases by comparing and extrapolating findings from human counterparts. However, despite biological and morphological similarities, the molecular pathogenesis of AS has been incompletely elucidated. This means that understanding the molecular mechanisms underlying HSA pathogenesis holds the potential to benefit not only dogs but also humans. Accumulating scientific evidence and data through basic research will lead to the identification of novel therapeutic targets for both HSA and AS.

Figure legends

Fig. 1 Frequencies of canine HSA and human AS in each organ.

Fig. 2 Gross images of a canine HSA case.

Labrador retriever, 9-year-old, castrated male. Multiple HSA lesions were found in the spleen (top), heart (middle), liver (bottom). Arrowheads indicate the HSA lesion of the right auricle of the heart. Bars = 2 cm.

Fig. 3 Histopathological and immunohistochemical images of canine HSA in the spleen.

(Left) Conventional type, Miniature Schnauzer, 9-year-old, castrated male. (Middle) Kaposiform type, Boarder Collie, 13-year-old, castrated male. (Right) Epithelioid type, Boarder Collie, 13-year-old, castrated male. The Kaposiform type and epithelioid type were obtained from different parts of the splenic mass in the same individual. HE: hematoxylin and eosin staining. CD31, vWF: immunohistochemistry for each antigen. Scale bars = 50 μm.

Fig. 4 Histopathological images of human cutaneous AS.

Representative images of human AS showing similar morphological patterns to canine HSA. (A) 82-year-old man. Similar to conventional type of HSA. (B) 72-year-old woman. Similar to Kaposiform

of HSA. (C) 84-year-old woman. Similar to epithelioid type of HSA. Scale bars = 50 μm.

Fig. 5 Histopathological images of a patient's tumor and PDX models of a canine hemangiosarcoma case.

Toy Poodle, 11-year-old, castrated male. P: passage. Scale bars = 50 μm.

Table 1. TNM classification of clinical stages in canine hemangiosarcoma.

Acknowledgements

No financial support has been provided for this review.

Conflict of interest statement

The authors declare that there is no conflict of interest in this study.

Ethics statement

AS patients and owners of HSA dog patients were thoroughly informed about the research aims and protocols, and written consent forms were obtained prior to the investigation.

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Fig. 1

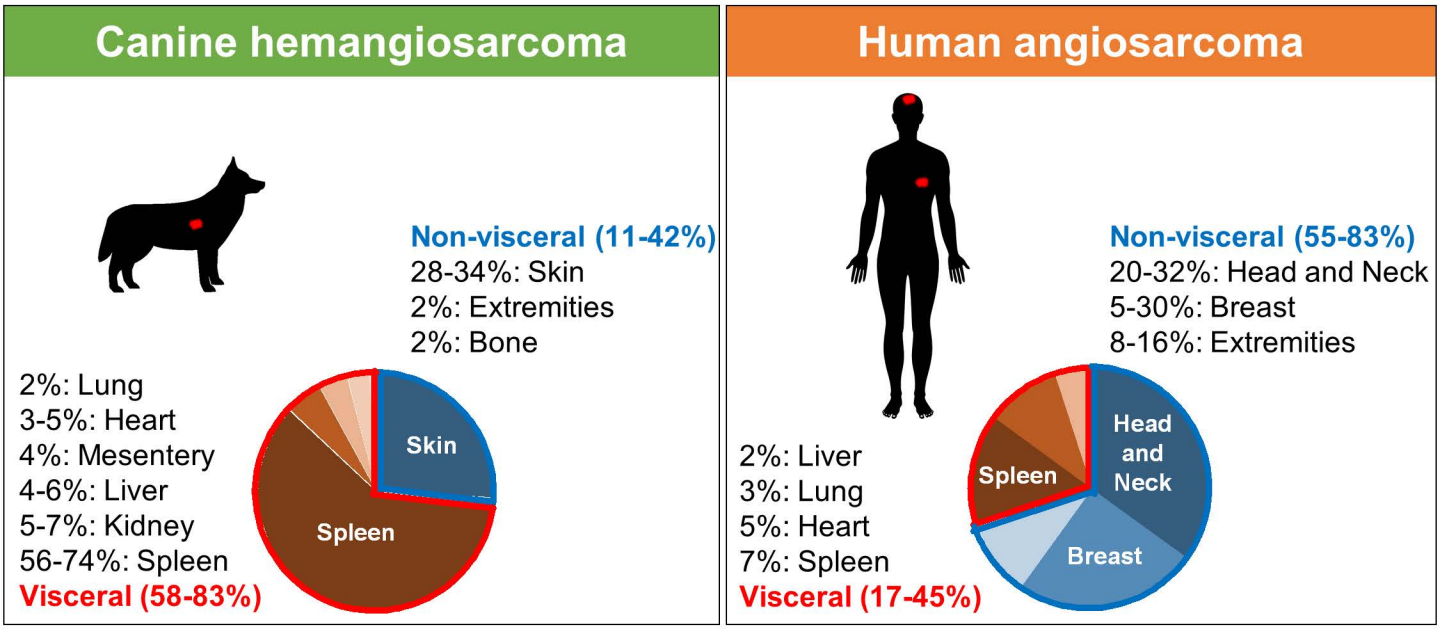
Canine hemangiosarcoma
Non-visceral (11-42%)
28-34%: Skin
2%: Extremities
2%: Bone
2%: Lung
3-5%: Heart
4%: Mesentery
4-6%: Liver
5-7%: Kidney
56-74%: Spleen
Visceral (58-83%)
Skin
Spleen
Human angiosarcoma
Non-visceral (55-83%)
20-32%: Head and Neck
5-30%: Breast
8-16%: Extremities
2%: Liver
3%: Lung
5%: Heart
7%: Spleen
Visceral (17-45%)
Spleen
Head and Neck
Breast

Fig. 2

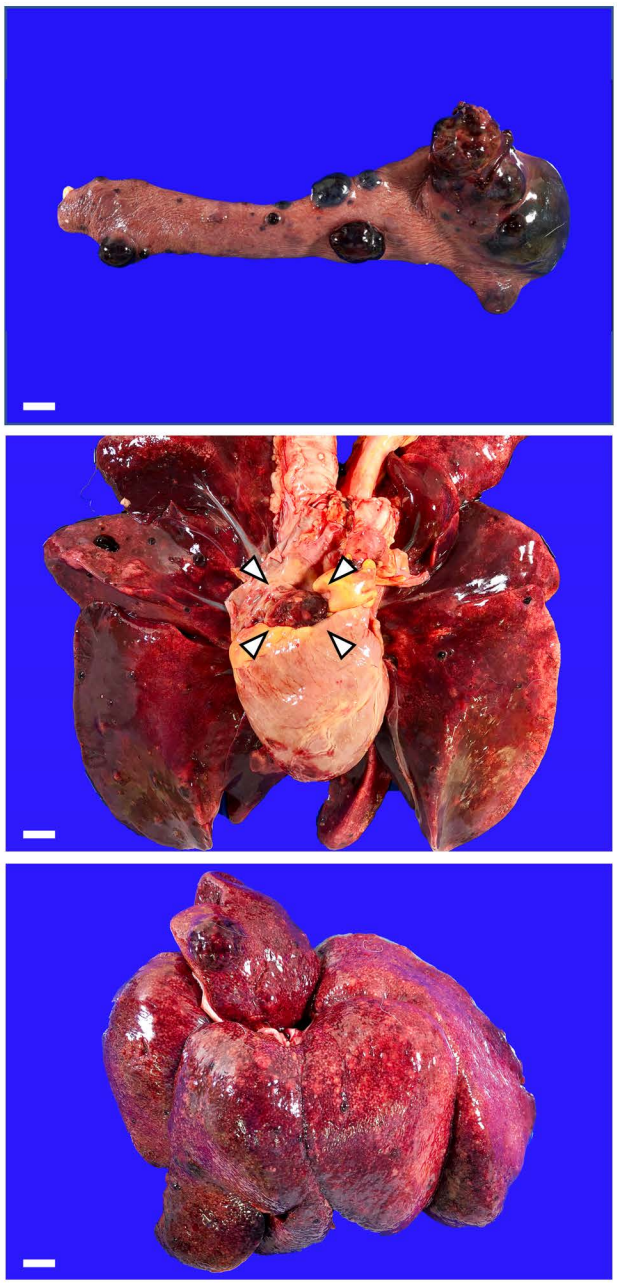

Fig. 3

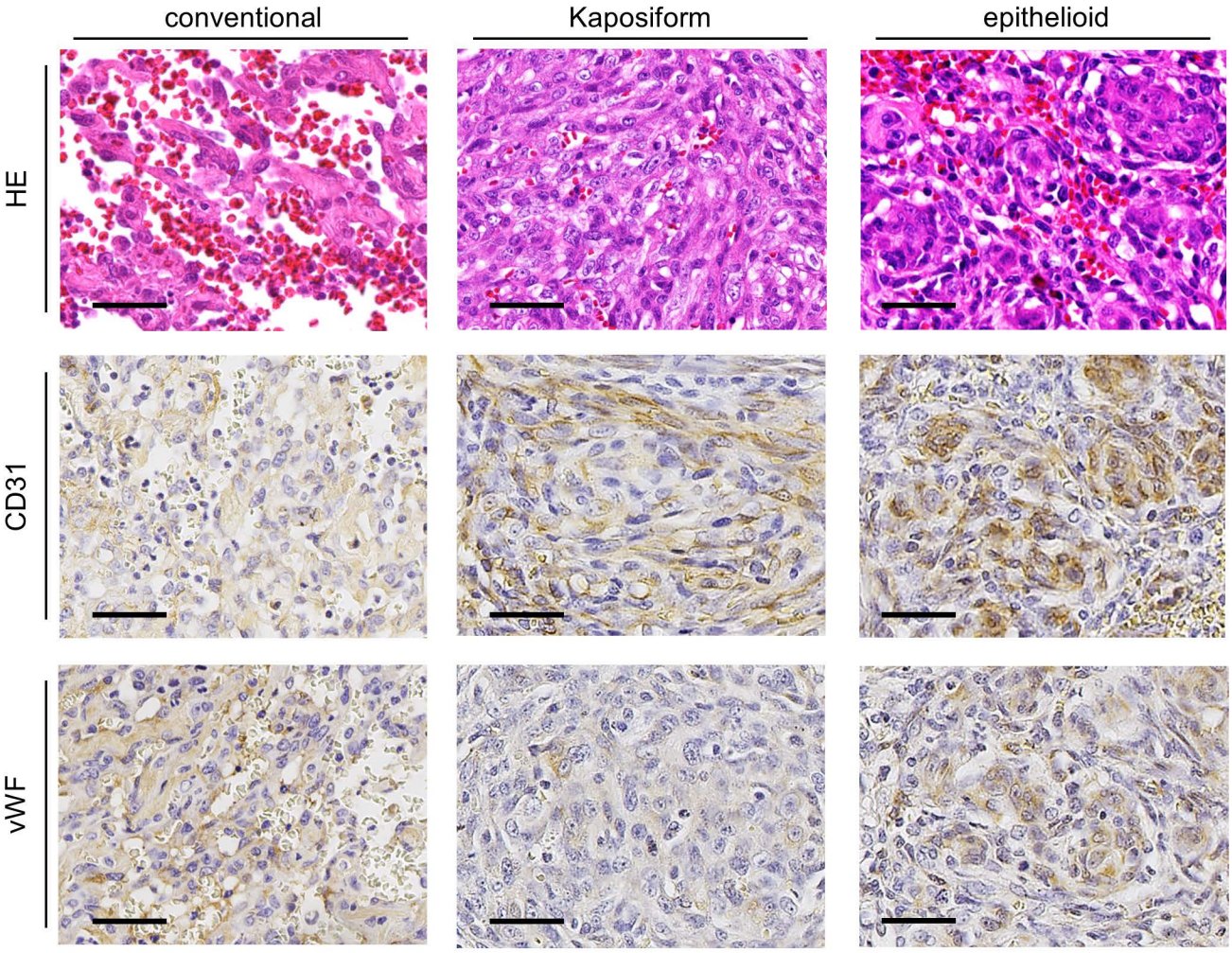

Fig. 4

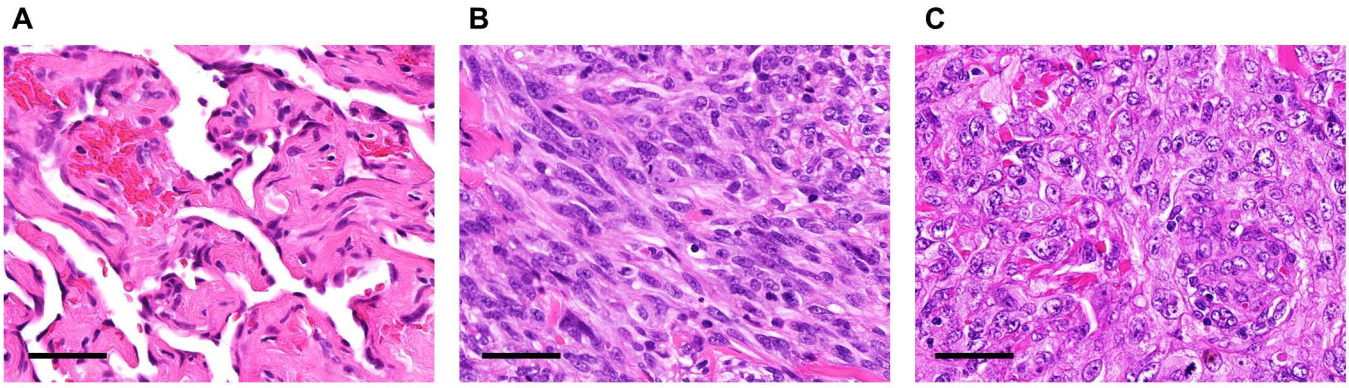

Fig. 5