Canine Hemangiosarcoma:
A Certainly Less Than Ideal, Very Ugly Cancer

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Abstract

Canine Hemangiosarcoma (HSA) is a devastating cancer affecting blood vessels in numerous sites within the body that is primarily seen in middle to older aged dogs. It is marked by its rapid aggressive metastatic pathology that often results in a lack of apparent symptoms in early stages. In most cases, disease becomes apparent due to hemorrhagic events following the rupture of the malignant vascular cell structures that can capture and pool blood cells, resulting in necrosis of the affected tissues. The poor survival times in affected patients cause a hindrance to the ability to carry out large scale studies, leaving numerous knowledge gaps to be filled in future research. The pathologic similarities between this and human angiosarcoma (HA) provides the potential for translatable research to be carried out that would improve outcomes across species. Here, current knowledge is outlined in order to improve understanding HSA holistically and suggest future direction. Emphasis is placed on the potential to improve veterinary practices in ways that will improve the ability to quickly and accurately diagnose patients in order to establish better client communication and provide clarity in collaborating to create the best informed treatment plan possible.

Keywords

Hemangiosarcoma, neoplasia, oncology, metastasis, doxorubicin, hemoabdomen

Introduction

Canine Hemangiosarcoma (HSA) is a highly aggressive metastatic cancer that develops from the endothelial cells responsible for creating blood vessels. As a relatively common form of canine cancer, HSA affects approximately 50,000 middle aged to older dogs in the United States each year.[1] Despite the overwhelmingly grave prognosis, involving mean survival times between 1 and 7 months [2](depending on treatment) and a 1 year survival time which remains generally less than 10%[3] while adhering to the recommended standard of care, there have been no significant advances over the past 20-30 years.[4] The last detection of efficacy signal in treatment of HSA, prior to a recent 2017 study, was in 1995 which goes to reinforce the need for advancements in care.[5]

Increased efforts to improve survival outcomes are worth the time to pursue for a few reasons. Emotional interests arise from appreciating the way dogs in modern society have come to fill numerous pivotal roles in their relationship with humans. These relationships, be it by serving a working role, or providing companionship that bolsters the emotional well-being of their human counterpart, have grown closer within the last 100 years. Not surprisingly the integration of dogs into our lives as members of the family has led to increased awareness of their afflictions including HSA, noting the

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first reports only date back to the 1950's and 60's correlating with the time that we began building these relationships.[6] Feelings aside, considerable justification for improving therapies is rooted in the prospective applications for HSA patients as models for building a deeper understanding of the pathologies driving human angiosarcoma (HA) and ultimately aid in developing novel experimental therapies. HA is a rare but deadly subtype of human soft tissue sarcomas (1-2%) [1]which is morphologically similar to HSA, consisting of tumors invading the endothelial layer of blood vessels and results in equally grim median survival times reported to be at around 16 months[7] . This cancer remains poorly understood due in part to its rarity leading to lack of access to patients for trials. HSA offers promising opportunities to advance treatment protocols for humans with HA for a number of reasons. Canines experiencing spontaneously developing tumors make excellent models because of their similar environmental exposures, favorable body size for imaging and serial biopsy sampling, shortened time course of disease progression, the development of treatment resistance seen in these cancers along with tumor burdens that are comparable to those seen in humans[8]. No immunocompetent mouse models of angiosarcoma are effective as models for studying immune based approaches to therapy.[9] Therapies that are able to be investigated within mouse models often lead to data that poorly translates to human application due to differences in immune status, drug distribution and metabolism, and achievable in vivo drug concentration differences. HSA cell line studies may also hold comparative potential in understanding other human cancers as they have been suggested to have originated from cancer stem cell lines similar to those seen in leukemias, brain tumors and mammary carcinomas[10].

Unfortunately, despite continued efforts, an overall lack of significant advancements have been seen in recent decades and knowledge gaps are apparent in nearly every aspect of this disease. My primary goal for this paper is to provide a brief, but comprehensive overview of the understood molecular and clinical implications of HSA and emphasize novel therapeutic designs.

HSA Subtypes

HSA cancers are divided into two subtypes, visceral (internal) and non visceral (cutaneous), depending on the location of the primary tumor. Comparison of canine tumor samples submitted to the Colorado State University Diagnostic Laboratory over a six year period of time identified visceral tumors making up more than half of the collected samples that were identified as HSA. No changes were seen in the general number of HSA cases over this time period.

HSA tumors have the potential to develop anywhere in the body, however they are most commonly found in the spleen (28-50%), right atrium/auricle (3-50%), skin or subcutaneous tissue (13%)[11]. When considering these percentage values, it may be worth noting the possibility of inflation regarding the reported occurrence rate for right atrium tumors as one study has determined 25% of splenic tumors had corresponding cardiac tumors that can be generally accepted as two primary tumors, rather than a primary tumor with metastatic site.[11] Both types have identified variations in specific breed overrepresentations. Greyhounds, Whippets and mixed breeds were the most reported in cutaneous cases of HSA[12], while German Shepherds, Golden Retrievers and Labrador Retrievers are most often reported in visceral cases[13]. Regardless of type, the same age group (median age 10) is most often affected in data that has remained consistent over time.[3] Less often reported is the finding that cutaneous HSA patients have been reported to be 1 year younger on average compared to those with visceral HSAs[13]. Not surprisingly, visceral HSAs tend to have lower median survival times and one year survival times when compared to cutaneous forms. Considering this in conjunction with the increased prevalence of this subtype, visceral HSAs generate more interest for research. Additionally, the desire to investigate visceral HSAs is influenced by how well these cancer cells in particular translate to application in human cancer studies.

While the prognosis remains variable based on the location and depth of invasion the tumor has achieved, studies have found cutaneous HSA may be resolved following surgery unlike what has been observed in cases of any visceral tumors[13], although chemotherapy regimes may follow surgical excision. [14]Identification of risk factors for cutaneous HSA, such as sunlight, which can be avoided in efforts to avoid cancer to begin with and prolong disease free intervals in patients who

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receive treatment contributes to further successful outcomes seen in these cases. The remainder of this paper will primarily focus on visceral HSA subtypes because of the greater need to improve successful outcomes in these cases.

Increased variability in pathogenesis, treatment options and overall outcomes that have been identified within visceral HSAs, yet even more remains misunderstood. In general, without treatment dogs diagnosed with internal HSAs will die within 1 to 2 weeks, although some may survive for months. [15] Splenic HSAs are the most common form and make up 45% of all splenic masses[2]. They have median survival times variable based on treatment protocol with surgery alone reported to be between 19-83 days and surgery followed by chemotherapy improving this time to be between 4 [15]and 8 months[14]. It is suggested that tumors originating in this site are more associated with metastasis even if no secondary tumors are apparent at the time of surgery making it incredibly rare for surgery to prove curative[3]. Cardiac HSAs are the most common type of cardiac neoplasia in dogs, making up 70% of cases[16], while remaining the second most common location for HSA primary tumors[17]. The prognosis is similarly poor compared to other visceral HSAs and involve the presence of distant metastasis in 75% of cases at the time of presentation.[17] These tumors also tend to present many obstacles in achieving successful surgical outcomes due to the increased difficulty of the surgery itself. While other sites have reported cases of visceral HSA, these locations are of primary interest in research.

Mechanisms of Action

Understanding the development, proliferative actions and metastatic nature of HSA cells remains of considerable interest across studies. The inability to improve diagnostics and treatment protocols is said to be hindered in part by knowledge gaps in understanding these cancer cells on a molecular level. Better insight into HSA cell line mutations, protein expression and pathway associations may further support the role HSA canines can serve as model systems for HA and other cancers in humans.

Cell Origin

HSA cells have long been presumed to originate from transformed endothelial cells.[14] However, until recently two competing hypotheses have competed regarding the specific origin of these tumor cells. To address this uncertainty, researchers aimed to determine whether HSA cells originate from differentiated vascular endothelial cells that undergo mutations leaving them malignant, or whether these cells are the product of transformed hemangioblastic stem cells which are incompletely differentiated cells from bone marrow at or near the stage of endothelial cell commitment[10].

Coexpression of surface markers associated with hematopoietic precursors with commitment to endothelial lineage including the observation of stable expression of CD146 and CD105 confirmed in 8 different HSA cell lines were identified. There is general consensus that mature endothelial cells commonly express the markers identified, along with VEGFR, CD31, CD14, CD34 and factor VIII-related antigen[18]. When considered along with previous findings that HSA cells represent primitive lineage based on their expression of C-Kit, it is likely that the hypothesis suggesting a bone marrow progenitor origin at various stages of hemangioblastic differentiation is supported.[6,10] The variable expression of CD45 and CD14 implicate may additionally prove to be valuable in targeted therapies. It is still unclear whether these cells originate from bone-marrow derived stem cells found in the vascular endothelial lining or from stem cells that are associated with blood vessels after transformation.

This information suggests potential for the capability to identify the presence of these cells in circulation while distinguishing them from normal or other malignant white blood cells. If HSA lesions do in turn shed endothelial progenitor like cells (EPC) continuously, diagnostic tools could be derived for use in early stage cancers to detect excess EPCs when evaluated alongside a reference value. Although EPCs can show increased values in conditions including vascular injury and cytokine influx, these values are known to remain below 0.5%[10]. Limitations worth considering

include small sample size limiting determinations regarding whether or not HSAs arising in different locations have different cell phenotypes and may warrant future investigation.

Further investigations into the tumor cells themselves include genome-wide expression profiling. This profiling has identified three distinct tumor subtypes based on the expression of genes, group 1 (angiogenesis) group 2 (inflammation) and group 3 (adipogenesis). The genes suggestive of subtype also was found to reflect the composition of the tumor microenvironment, and potentially the balance of hypoxia and inflammation observed during tumor formation[6]. Inflammation along with angiogenesis are recurrent features of HSA cell lines that have been shown to express functional receptors initiating biologically relevant signals upon binding IL-8, CXCL12 and modified sphingosines.[6] The identification of specific tumor subtypes suggest a common HSA progenitor may be responsible for these distinct differentiations and may indicate a capacity for dynamic evolution within tumor cells.[1] The identification of potential subtypes may provide a direction to allow further understanding tumor cells and their microenvironments[6]

Identified Mutations

Unlike what has been described in cases of other sarcomas, HSA tumorigenesis may be driven by small insertions and deletions and single nucleotide variants rather than high-grade alterations at a chromosomal level. In a study designed to identify oncogenic drivers, researchers implemented the first use of newly developed canine exome capture reagents to sequence HSA tissue samples.[1] Data collected identified somatic mutations in PIK3CA, TP53, PTEN, PLCG1 which correspond with known tumor drivers associated with human cancers in half of the samples analyzed. While no consensus was seen across samples that would result in a level of confidence in identifying a driver candidate, mutations in PIK3CA was considered a likely candidate as it was the most frequent pathway affected by mutations (45% of cases) (48.9%) and shares 99.8% homology with human cancer gene mutations[19]. Cell viability assays revealed significant reductions by PI3K inhibitors, which have also been demonstrated to slow growth of primary HSA cells derived from both visceral and cutaneous HSA tissues. TP53, found to be the most frequently mutated gene, was also identified as having several functions affecting DNA binding domains. TP53 has also been noted in certain human cancers.

Further, research revealed a predicted activating mutation in PLCG1 in one sample, which is homologous to mutations in HA. S273 is a highly conserved protein in the kinase domain of PLCG1 known to exhibit enzyme activation and increased downstream signaling of T cell receptors and NF-Kb. This suggests PLCG1 S273 may contribute to pathogenesis and might be representative of a subgroup of HSA with great molecular similarity to HA, explaining why it was only seen in 1 of the above cases. Although more genes in this study were identified to have mutations, analysis did not reveal sufficient evidence to support a role in pathogenesis.[1]

Building on this previously completed work, more recent efforts focused attention on obtaining improved genome wide data by completing the largest exome sequencing study to date in order to allow for more comprehensive comparison with HA. Somatic coding mutations were again identified as occurring most frequently in Tp53 (59.6%) and on 2 genes in the P13K pathway consisting of the oncogene PIK3CA and the regulatory subunit PIK3RI.[19] The most predominantly noted mutational signature was identified as the age-associated deamination of cytosine to thymine which was present across all HSA tumors regardless of location or tissue preservation method. This project also failed to identify specific driver genes, but has suggested additional possibilities in the RASA1 and ARPC1A genes. RASA1 has a role in vascular formation as a negative regulator of RAS and the MAPK pathway and germline mutations in this gene can lead to capillary malformations. ARPC1A plays a role in regulating actin in cytoskeletons in addition to known functions in migration and invasiveness in pancreatic cancer cells.

Two key differences across species have since been identified using expression of markers for hematopoietic stem cells, endothelial cells, a tumor suppressor protein and a myeloid marker in canine HSAs. First, most HA cells had HSC expressions indicating they were made up of tumor cells that were less differentiated than those in HSA. The second

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noteworthy difference was identification that HA and HSA cells expressed late-stage endothelial cell maturation markers. Similar tumor cells in mice were negative for these markers leading to the belief that HA and HSA tumor cells may have greater differentiation potential than mouse tumors.[18]

Metastatic Process

The advanced metastatic nature observed in HSA is a complex process early and widespread process with multiple factors leading to success.[20] This process that may occur either hematogenously, spreading by blood circulation, or by local seeding methods following tumor rupture[11]. The most common sites for metastasis are typically the liver, omentum and lungs, but as with primary sites, they are not limited to these locations. Similar to what was described regarding cell origins and expression markers, much of the pathways utilized in metastatic processes remain poorly understood. While many possibilities have been explored in research, I will focus on a few that appear to me implicated most frequently.

One pathway involves the likelihood that HSA the somatic point mutations discussed in the previous section upregulate PI3K, which plays a role in signal transduction that leads to cell proliferation, survival, differentiation and the regulation of both immunologic and metabolic functions[1]. This pathway is one of the most commonly altered pathways in many forms of cancer. [19]PI3K inhibitors were shown to slow growth of primary cells from both forms of HSA providing additional support for the potential that driver mutations may affect this pathway significantly.

CD18+ inflammatory monocytes are found in significantly larger numbers in HSA mets than in mets from any other tumor type and form uniquely dense infiltrative patterns 3-5 layers thick within HSA that are predominantly localized to fibrous connective tissue stroma and the connective tissue bundles which form vascular spaces, rather than within the vascular spaces themselves. In comparison, CD18+ cells more frequently found in small to medium nodule clusters randomly scattered throughout the periphery and within the tumor core in both soft tissue sarcomas and osteosarcomas or localized within bands of reactive fibrous connective tissue in individualized, less dense patterns as seen in melanomas and transitional cell carcinomas in dogs. Pulmonary mets found in HSA patients have capabilities to recruit these monocytes to a significantly greater degree than other canine tumor mets which has been experimentally confirmed by means of antibody mediated cell depletion, which prevented metastasis of tumors in mice. [20] Inflammatory monocytes, which can be defined by high surface expression of CCR2, promote metastasis by stimulating tumor cell evasion, extravasation, seeding, growth and angiogenesis. HSA cells were found to produce the monocyte chemokine CCL2, which is the primary ligand for the CCR2 molecule, in larger numbers than other cancer cell types. The chemotactic axis of CCL2-CCR2 is therefore able to stimulate the recruitment of inflammatory monocytes to secondary sites where they can differentiate into metastasis-associated macrophages (MAMs) which prepare the microenvironment to welcome tumor cells. The metastatic microenvironment becomes favorable for the establishment of newly recruited tumor cells by the production of cytokines and growth factors, in particular vascular endothelial growth factor (VEGF) by MAMs. Previous studies have indicated high serum CCL2 concentration along with elevated circulating monocyte counts as negative prognostic factors in human cancers which may provide similar indications in looking at HSA. The strong infiltrative patterns seen could suggest why poor response to chemotherapy is typical. Understanding the influence of monocyte recruitment in disease progression provides indications for the investigation into how blocking this process may serve as an effective strategy for suppressing the metastatic process.[20]

While these findings may provide some answer as to the aggressive metastatic nature of this cancer, it may not be the sole contributor to success. Previous studies investigating the role of the CXCR4/CXCL12 chemotactic axis determined *in vitro*, CXCL12 promoted calcium mobilization, cell migration and invasion directly proportional to CXCR4 surface expression on in HSA cell lines which were responsive to the use of AMD3100, a CXCR4 antagonist[21]. CXCR4 is likely the most common chemokine receptor seen expressed on tumor cells and serves as the primary receptor for CXCL12, which is expressed in lymph nodes, lung, liver, spleen, heart and in bone marrow.[21] This interaction was observed in the

use of HSA primary cells as opposed to the CCL2-CCR2 investigation which used pulmonary metastatic tissue samples, which may indicate the significant players vary depending on the stage of metastasis. Still, it remains unclear whether HSA cells create CXCL-12 enriched environments which affect CXCR4-expressing cells of if CXCR4 cells migrate to and colonize organs where there is existing abundance expression of CXLC12[6]. If this is the case, either chemotactic axis may prove to be an attractive option for targeting in the development of new therapies to prevent metastasis depending on disease progression.

Clinical Findings

With some foundation-level understanding of the cancer's molecular processing we can move into investigating what this complex process actually looks like in more detail. Veterinary practitioners who face these cases first hand should have a level of confidence in not only their ability to diagnose these patients, but also to accurately convey prognostic variables in their client communication. The process of informed decision making on the owner's part can be challenged by the sudden and often urgent presentation of HSA in their pet's. Emphasis on early detection has been recognized as being critical to improving outcomes, but is not often a possibility. Because of this a thorough understanding of current diagnostic capabilities and treatment options becomes increasingly valuable for practitioners in their effort to improve client communication in these cases.

Presentation

HSA is often asymptomatic prior to the day of presentation. In patients who do exhibit symptoms may or may not include unexplained weight loss, bulging of the abdomen, decreased exercise tolerance, lethargy, decreased appetite, pale mucous membranes, cough or collapse. Some pet owners may describe histories including bouts of weakness in the recent past that resolved without intervention over the course of 24 to 48 hours. In other cases a more chronic history of weakness, lethargy or inappetence may be reported. [3] Clinical signs in patients with HSAs most often occur on an emergency basis as a result of acute hemorrhage from the tumor[2,6,17]. Many patients with splenic HSA present with hemoabdomen following mass rupture[22]. Hemoabdomen is the result of hemorrhage frequently associated with splenic HSA tumors and results in an accumulation of blood in the peritoneal cavity. Patients experiencing this may become anemic and experience secondary symptoms resulting from decreased oxygen delivery to tissues that results, as is often the case of patient collapse.[23] While splenic rupture is the most commonly discussed hemorrhagic event associated among HSA types, it is worth noting that as many as 90% of dogs with HSA experience one or more hemostatic disruptions implying the need for thorough examination to ensure bleeds are properly managed. Management of these patients may include the need for blood products which require further consideration into transfusion reactions in the cancer patient in addition to the additional costs and monitoring these measures would require.

The sudden onset of symptoms is also reported in HSAs originating from other sites. For example, a 6 year old wheaten terrier mix with metastatic cardiac HSA, displayed decreased energy levels for 1 week prior to admittance for collapse and lethargy following vomiting up a large amount of clear fluid. Despite the advanced state of his condition, he was only mildly dyspneic, but showed no signs of true respiratory distress. Upon closer evaluation, tachycardia was noted along with muffled heart sounds when auscultated, implicating the need for further diagnostic evaluation. Radiographs and ultrasound revealed pericardial effusion, enlarged cardiac silhouette, diastolic collapse of the right atrium and an identified right auricular mass, but no obvious signs of metastasis. It wasn't until surgical exploration that several metastatic masses were seen in the pericardium and were also removed.[16]

Another case study revealed a similar pattern of nonspecific gastrointestinal symptoms with no apparent cause in a daschund with primary colonic HSA[24]. Following two previous visits, each of which consisted of diagnostic screenings with no significant findings reported either time, the patient represented in critical condition with decreased mobility, increased melena and hematochezia, pale mucous membranes and weak femoral pulses. Bloodwork at this visit showed

severe anemia, thrombocytopenia, hypoalbuminemia and hypoalkemia warranting the need for an emergency exploratory surgery which revealed a mass on the colon confirmed to be HSA.

Diagnosis

The common occurrence of patients presenting with nonspecific and variable symptoms as described above present practitioners with diagnostic challenges. In many cases that are examined in likely early disease stages, diagnosis can be easily missed. As was the case in the colonic HSA patient who despite thorough screenings including bloodwork, radiographs, ultrasound and endoscopy, remained undiagnosed until she became critical. With early diagnosis being one of the few variables leading to somewhat favorable outcomes in patients, advancements that could improve this process are highly desired.

HSA is definitively diagnosed by pathologist review of biopsies collected via surgery, as fine needle aspirates do not typically yield accurate results[3]. Due to complications caused by necrosis and hemorrhage, while there is no official consensus about the number of sections that should be evaluated, an effort to determine this was carried out using Monte Carlo simulations following the manual review of two boarded pathologists which determined a 95.02% chance of diagnosing HSA resulted from the submission of 5 samples, whereas the evaluation of 10 samples yielded a 98.59% chance[25]. With these numbers determined, five section samples are considered likely sufficient in evaluating submitted specimens. While it remains recommended to submit an entire removed specimen in order to determine the completeness of excision, the identification of these confidence levels help establish better likelihood of accurate diagnosis.

Imaging tools including abdominal ultrasound, radiographs including multiple views, or CT scans can be useful tools in determining the extent of disease present, but should be evaluated with caution as signs of metastasis may still be missed using these measures. Rather than providing clear evidence of HSA presence in the body, radiographs often provide clues that require further investigation including loss of detail due to effusion, or mass effects resulting in mispositioning of visible organs. [12]Positron emission tomography- computer tomography has been suggested as a better diagnostic tool for identifying these signs, but may not be widely available to most general practitioners.

A complete blood count (CBC) is highly stressed in accurately assessing patient condition in dogs presenting with suspected HSA to identify signs of regenerative or nonregenerative anemia with or without thrombocytopenia as these are common observations in the advanced disease process. If the patient is experiencing a hemorrhagic event, fluid collected should reveal bloody effusion liquid that fails to clot. Tumor cells may or may not be present in this sample, but are often too difficult to detect microscopically due to the large quantity of blood cells present. While flow-cytometry based screenings can be performed on these samples that may allow for the detection of circulating HSA cells in the blood, this is also not a tool available for use in general practices.

These limitations emphasize the potential value in the ability to determine the presence of cancer cells in critical patients prior to pursuing surgery. At this time reaching a definitive diagnosis is rare prior to surgery, which places a significant burden on the practitioner and pet owner alike in making the ability to make the best informed decisions possible regarding the pursuit of care.

Currently, no confident blood test for HSA is available, although there have been indications, as mentioned in this review, that some tumor associated cell markers may lend well to valuable screening tools due to their unique overexpression within HSA cells. The variability seen in subtypes of HSA cells create the possibility for a multitude of new screening measures specific to the type of interest. For example, cardiac troponin I (cTnl) blood tests, which are currently available for detecting heart attacks in humans, is designed to measure the level of cTnl protein which is released into the blood when the heart muscle is damaged [26]and have been suggested for use in allowing the distinction between hemopericardium from cardiac HSA and other idiopathic events.[2] Alternatively, interest has been expressed in the

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potential for the use of TK1, an enzyme important in DNA replication, as a marker for screening due to known elevated levels seen in a majority of HSA patients.

Prostate specific membrane antigen in humans PMSA is a transmembrane protein that is overexpressed in prostate carcinomas and tumor associated endothelium of additional cancers.[27] It has previously been identified as being expressed in HSA cell lines in qualitative studies which warranted further investigation to quantify this expression. PMSA could not be identified in circulating whole blood samples from healthy dogs, but were detectable in both hemorrhagic effusions from dogs with cHSA and PMSA expressing cancer samples. Upon quantification, researchers identified PMSA gene and protein expressions to be increased up to 6 fold in cHSA compared with samples from non-malignant endothelial tissue. One limitation noted in the use potential of PMSA detection is while it is overexpressed in HSA compared to normal samples, the expression of PMSA is not solely restricted to tumor tissues and has been identified in some normal visceral organs. Despite this potential, this recognized overexpression remains of considerable interest for the development of molecular imaging reagents for diagnostic purposes as well as targeted therapeutics.[27]

Treatment

As previously mentioned, the prognosis for HSA in canines is most often poor despite the course of treatment pursued due to the level of cancer progression that is typically achieved prior to diagnosis. As is the case with early detection, prognosis for patients is improved prior to tumor rupture due to the likelihood for peritoneal and omental metastasis resulting from tumor seeding.[8] In cases with noted gross metastasis at the start of treatment, reported outcomes involve only a 40% response rate and response duration of 53 days.[8] Most cases of HSA are managed with surgical excision of the primary tumor along with any apparent mets followed by chemotherapy protocols. Due to the continued shortcomings in this approach, many potential therapies have been investigated in the hope of identifying ways to improve overall survival times as well as prolong remission in patients. While these efforts remain ongoing, a few studies have yielded results that provide improved direction for future therapies.

Current standards of care

Splenic HSA is the most common form and therefore has the most investigation regarding treatment efficacies. The standard recommended protocol involves splenectomy followed by doxorubicin (DOX) chemotherapy to be given once every 2-3 weeks intravenously for a planned minimum protocol of 5 treatments, or until disease progression is noted[17]. DOX is an anthracycline based drug that works to slow the growth of cancer cells by blocking topoisomerase 2 and is the preferred drug of choice due in part to relatively low toxicities, and low cost making it accessible to most patients[28]. In a retrospective study, the evaluation of this protocol yielded similar common clinical signs in both treated and untreated dogs which included lethargy, weakness, collapse, coughing or respiratory difficulty, anorexia and vomiting. CBC abnormalities were also similar between groups of dogs and commonly indicated neutrophilia, anemia and thrombocytopenia. Median progression free (66 days) survival and median survival times (116 days) remained short, but improved. While this study did not mention the influence surgery has on outcomes, a previous study using the same chemotherapeutic protocol has shown surgery outcome to markedly influence survival time with 20% of patients alive at 1 year who experienced complete excision, compared to no survival for patients with incomplete excision at 1 year.[14]

Few large scale studies on the management of cardiac HSA exist in comparison to those performed looking at splenic HSA.[17] Difficulty with local control of cardiac tumors result in decreased feasibility of resection, which depends heavily on the size of the tumor, restriction of tumor to right atrium or atrial wall, coronary artery and heart valve involvement and the ability to create a pericardial patch graft if wall resection is required. Pericardiectomies, which involve removal of pericardium tissue, have better outcomes in non-vascular heart tumors and are a palliative approach that is a possible effort if resection isn't possible. Due to these limitations affecting surgical management of cardiac based HSA, chemotherapy is generally the recommended primary therapy.[3]

Combination therapy

Combination therapies have gained interest in research and are designed to overcome continued poor survival times, adverse events associated with toxicities and the formation of drug resistance that are often seen with DOX treatment. While mechanisms of drug resistance is not yet fully understood, multiple studies have identified an increased drug efflux pump capacities in HSA cells resulting from increased expression of drug transporters mediating drug efflux along with increased efficiency in DNA repair[4] via a decrease in damage response[29] as being likely drivers to avoidance mechanisms.

These protocols often consist of existing drugs that have been shown to work in other canine cancers or human cancers and are designed to maximize efficiency in inhibiting cell growth or create hostile microenvironments. One study investigated a protocol using doxorubicin, dacarbazine and vincristine (DAV) in dogs with nonresectable HSAs[30]. Dacarbazine is an alkalizing agent suggested to have synergistic action when used in combination with anthracyclines (DOX) and has shown to be effective in some high grade sarcomas in humans. Vincristine is also a growth inhibiting drug that stops cells from dividing and has been previously studied for use in other combination therapies (with and without DOX and other agents)[11,12,14,30] for treating both cutaneous and noncutaneous HSAs with modest results in a limited number of those cases[28]. The reported response rate following this therapy was 47.4% with a median time to progression of 101 days and MST of 125 days with dogs showing complete remission having disease free intervals of 205 days. These findings appear comparable to the previously defined standard doxorubicin treatment, a key difference in this study being the use of patients with gross metastatic disease at the time of treatment initiation which provides indications for favorable outcomes in advanced staged patients than previously considered.

Multiple combination therapies have been investigated and have yielded more questions than answers. A recent report describes the efforts investigating combinations of existing drugs, immunotherapy and new drugs over the last 20 years as showing no benefit in improving overall survival when compared to the current protocol[15]. Reviewing the favorable outcomes that have been suggested in some studies, this is likely due in large part to the limitations presented in these studies which included small sample sizes, additional interventions outside the scope of the study, inaccurate reports or lack of owner compliance in completing studies and omission of patient data that may prove valuable due to circumstances that arise during the study. In order to better appreciate the effects of modified chemotherapy treatments, future studies should aim to expand their sample size while ensuring more stringent adherence to protocols.

Novel Approaches (dendritic cell vaccination, Tenovin-6, Resveritrol, eBAT)

New delivery systems consisting of inhalation or intracavitary chemotherapy have been designed with the aim of inhibiting pulmonary metastasis. [11]Antimetastatic agents primarily targeting the inhibition of angiogenesis, the formation of new blood vessels from existing microvessels, is a favorable target in the metastatic cascade as this is the main route enabling tumor cells to leave the primary tumor site into circulation. There are four described strategies in designing antiangiogenic therapeutics: blockage of factors stimulating formation of new vessels, utilizing natural inhibitors of angiogenesis, blocking molecules that allow the newly informed vessels to invade surrounding tissue and the incapacitation of newly dividing endothelial cells. These agents appear unlikely to induce drug resistance, and have no reports of adverse effects associated with chemo, but do present the disadvantage of only targeting new vessel formation rather than pre-existing tumor tissues.[11]

Interferons alpha and beta are well recognized angiogenic inhibitors in human medicine. IFn2a inhibits angiogenesis by suppressing basic fibroblast growth factors and VEGF production which are elevated in cancer cells. IFn2b has also been of interest in studies for use in conjunction with regular chemotherapy. A reported shortcoming to pursuing this approach is the expense and challenge in ensuring owner compliance with a daily injection protocol. Additionally, there is potential for resistance and/or adverse effects resulting from the production of neutralizing antibodies to IFN overtime.

Thalidomide is another angiogenic inhibitor that is suggested to inhibit VEGF that was evaluated in clinical studies as a single agent in dogs that was reportedly well tolerated.

Oncolytic viral therapy has been considered for use in vascular tumors[31]. In a study which used a new recombinant MVMp/IP-10, inhibition of progression was observed in established H5V cell induced vascular tumors which was implemented for use as a model system for human cancers. MVMp is a parvoviral vector, selected for use due oncotropic/oncostatic features as well as the poor expression of these viruses in quiescent cells and most non transformed cells. IP-10 is an antiangiogenic agent. The autonomous parvo viral vector, MVMp did not have significant effects under the same conditions, but demonstrated strong antiHSA activity when used to infect H5V cells ex vivo prior to implantation in the host. The viral induced suppression of HSA was determined to be dependent on host T cell and intratumoral presence of IFNg expressing cytotoxic lymphocytes. This immune mediated response led to reduced expression of a metastatic marker, hepatic plasminogen activator inhibitor 1 (PAI-1). T cell involvement was confirmed by the use of T cell KO mice who were unable to form a response. This therapeutic design showed effects on hemangioma, but treatment of hemangiosarcomas with the vector alone proved unsuccessful suggesting specificity of IP-10 interference in cancer cell progression. While continued work will be required that focuses on the goal of proving the therapeutic potential for treating HSA cases specifically, this case showed the viral therapy was well tolerated and survival times were noted as doubling with the administration of doses significantly lower than are required for use with adenoviral vector therapies.

Dendritic cells are the queen of regulators in the adaptive immune response and have drawn attention for use in a therapeutic vaccine protocol designed for use in conjunction with recommended chemotherapy following surgical tumor removal.[9] A pilot phase I open-label trial which used this model of chemo-immunotherapy was limited by small sample size likely leading to a lower MST, 109 days, than may be achievable on a broader spectrum. Of the five animals who completed the course of treatment, one passed away 16 months later and was determined to be cancer free at the time of death. Based on these initial results, and the success described with more significant MST improvement when used in other cancer types, I believe results in broader HSA trials could see similar marked improvement.

Finally, one of the biggest breakthroughs in advancing HSA therapies involves the use of bispecific targeting of cell surface markers using eBAT.[15] eBAT is a targeted toxin from deimmunized pseudomonas exotoxin, which leads to the inhibition of protein synthesis, that is fused to human EGF and the amino terminal of urokinase. Human amino terminal transferase (ATFs) are the high affinity binding moiety of the urokinase that targets urokinase plasminogen activator receptor (UPAR), a GPI anchored cell surface protein that is associated with tumor invasion, migration and metastasis. In a trial with stage I-II splenectomized dogs given one cycle (3 administrations) of eBAT followed by delayed administration of doxorubicin, 6 month survival times were shown to nearly double[5]. Excitingly, 6 of the 23 dogs who completed the study experienced survival greater than 450 days despite the delayed administration of chemotherapy, which has previously been indicated to lead to poor prognosis. Adverse events were reduced from those previously seen in EGFR targeted therapies that are suggested to be in part to the targeting specificity to tumors as a result of the addition of the uPAR-directed ligand. As a proof of concept study, results were limited by the use of a comparison group as opposed to a true control group. Additionally, patients treated were candidates with minimal residual disease which may have led to the better outcomes observed to those patients in the comparison group.

This previous success was further built upon in this study by determining the effects of repeated cycles of eBAT along with a shortened duration to the start of chemo.[7] Unlike the previous work, no significant survival benefit was seen when comparing study patients to a comparison group. Increased toxicity and reduced efficacy was noted which warrants a deeper look into understanding the precise mechanism of action in eBAT therapies, which remains unknown. Improving our knowledge of this mechanism will hopefully allow future work to determine the best recommended protocol. Reasons for the decreased efficacy seen in this trial are not exactly known, but suggestions involve possible immunogenicity from

neutralizing antibodies against eBAT that led to a negative influence on tumor response, or the earlier administration of doxorubicin following eBAT administration may have suppressed the immunomodulatory effects of eBAT. Due to the increased toxicity noted in this trial, my hypothesis leans towards option A.

Future Goals

Many projects are continuing to advance diagnostic and treatment protocol in HSA patients. Efforts to further translate HSA studies to HA have called for analysis of all currently available Human Angiosarcoma data available through the Human Angiosarcoma project in order to decipher potential molecular subtypes for a more complete comparison between tumors in different locations[19]. Ethos veterinary health completed a nationwide, multicenter study on HSA (CHAMP) with the expectations to change misconceptions in the vet community and help owners make better decisions regarding treatment[32]. Studies are continuing to develop a molecular diagnostic tool that will help define prognosis and identify molecular derived treatments for unique suspected outcomes. Similar collaborative efforts are underway with the help of The Comparative Oncology Trials Consortium (COTC), a network of more than 20 academic veterinary oncology centers managed by the comparative oncology program within the NIH NCI center.[8] This network is improving the ability for potential research sponsors and collaborators to access national cooperative group for conducting proof of concept studies in dogs. These sponsors work with management to develop protocols aimed to address specific questions that are put out to members for participation opportunities. Other ongoing large-scale longitudinal studies include projects currently aiming to identify risk factors for cancers in golden retrievers, a clinical trial to evaluate the ability of a multivalent frameshift vaccine to delay or prevent cancer onset in older dogs, and determining incidence and risk factors for a variety of age related illness by profiling and following 10,000 dogs[8].

One area I would be interested in determining the efficacy of elective splenectomies in dogs to avoid HSA in this area. This is an idea I have heard discussed while working in the veterinary field, but failed to identify verifiable research on. Questions that would need to be addressed in this case would surround defining the criteria for recommending pets to pursue this as well as preparing for potential immunologic defects that would arise from removing the spleen in dogs.

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