

CANINE HEMANGIOSARCOMA - THE ROAD FROM DESPAIR TO HOPE

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In following article, we describe the current state of knowledge for canine hemangiosarcoma, including what it is, why it may happen, and how it can be managed. In addition, we present recent findings from our programs that promise to help us improve our ability to diagnose, treat, and prevent this disease.

The Natural History of Canine Hemangiosarcoma

Canine hemangiosarcoma is among the most challenging and mysterious diseases encountered in veterinary practice. It is an incurable tumor of cells that line blood vessels, called *vascular endothelial cells*. Hemangiosarcoma is relatively common in dogs; it is estimated that this type of cancer accounts for 5-7% of all tumors seen in dogs. Considering the lifetime risk of cancer for dogs is between 1 in 2 and 1 in 3, we can calculate that 1.5 to 2.5 million of the ~72 million pet dogs in the United States today will get hemangiosarcoma and succumb from it. Although dogs of any age and breed are susceptible to hemangiosarcoma, it occurs more commonly in dogs beyond middle age (older than 6 years), and in breeds such as Golden Retrievers, German Shepherd Dogs, Portuguese Water Dogs, Bernese Mountain Dogs, Flat Coated Retrievers, Boxers and Skye Terriers, among others. According to the Golden Retriever Health Study published in 2000, the estimated lifetime risk of hemangiosarcoma in this breed is 1 in 5, illustrating the magnitude of this problem.

Unlike other cancers, hemangiosarcoma is almost an exclusive disease of dogs. In people, a similar type of tumor (angiosarcoma) occurs only rarely in association with workplace exposure to vinyl chloride and polychlorinated biphenyls (PCBs), such as is found in rubber and tire plants. An even smaller fraction of women who receive high dose radiation therapy for cancer (usually breast cancer) can develop angiosarcoma of the skin. Yet, angiosarcomas account for much less than 1% of all tumors seen in people. Certain mutations are known to predispose laboratory mice to hemangiosarcoma, but whether these mutations contribute to the disease in dogs is not known. Benign tumors of vascular endothelial cells, called hemangiomas, arise in the skin of people and dogs that have extended exposure to sunlight. These tumors are distinct from hemangiosarcomas and angiosarcomas, and they are not life threatening. In dogs, the common primary sites for hemangiosarcoma are the spleen, the right atrium of the heart, and the subcutis, which is the tissue beneath the skin. The pattern of growth for these tumors involves infiltration into normal tissues surrounding the tumor as well as distant spread (metastasis). The disease is indolent; in other words, it does not cause pain and the rate of growth in the early stages is relatively slow. Dogs harboring even large hemangiosarcomas may show no clinical signs or evidence that they have a life threatening disease. Generally, the tumor cells retain some normal aspects of behavior, so they try to make blood vessels. But these vessels are tortuous and malformed, and blood cells tend to pool in them and clot. The clots then prevent blood and nutrients from reaching tumor cells, in turn causing them to die. This creates small ruptures in the tumor through which blood may escape into the abdomen, heart sac, chest, or subcutaneous space. Depending on the amount of blood lost, affected dogs may show non-specific (constitutional) signs such as lethargy and weakness, but these are transient and resolve as dogs reabsorb the blood components and make new blood cells. The clinical signs are recurrent, but they also are subtle enough to go unnoticed for some time. Since hemangiosarcoma tends to metastasize aggressively to lungs, liver, intestines, and mesentery (the membranous connective tissue that supports the intestines), distant spread (either microscopic or macroscopic) has inevitably occurred once the disease is finally diagnosed. The eventual outcome for patients with this disease often follows the rupture of a large or rapidly growing tumor, which results in acute, severe hemorrhage, collapse, shock, and death.

What Causes Hemangiosarcoma

We do not precisely know what causes canine hemangiosarcoma. The observations that the disease occurs more commonly in dogs than in other animals, and that some breeds are at higher risk than others tell us that heritable factors must contribute to risk. Ultimately, the interactions of these heritable risk factors with the environment probably lead to the spectrum of mutations that give rise to the tumor. As a matter of brief review, it is important to understand that "*cancer is a genetic disease, although it is not always heritable.*" Tumors arise when cells accumulate mutations that eliminate normal constraints of growth and genetic integrity. These mutations provide cells a selective growth advantage within their environment, essentially the same evolutionary phenomenon that we call

natural selection, albeit on a microscopic scale. Most mutations arise because the enzymes that control cell division are not foolproof. About 1 mutation occurs for each one to ten million bases that are replicated when time a cell divides (genomic DNA in dogs consists of about 2.5 billion base pairs; in other words, when a cell divides, it must copy 2.5 billion bases and the inherent error rate of the DNA replication machinery will introduce between ~250 and 2,500 errors, or mutations, to the DNA of each daughter cell). Since some cells in the body divide continuously to replace others that die or are damaged (for example, blood cells, skin cells, and gut cells), mutations are pretty much introduced constantly into cells in the body. For this reason, we say the greatest risk factor for cancer is "being alive". As mentioned above in the example of humans that are at risk to develop angiosarcoma, mutations also can occur from exposure to environmental toxicants. Fortunately, most of these mutations are silent (they neither help nor hurt the cell or the organism), and the body has mechanisms to eliminate most cells that acquire deleterious mutations. A relatively new concept about how cancer happens invokes the theory that only "stem cells" can give rise to tumors, making the acquisition of mutations in somatic cells (any cell that is not a sperm or an egg) a lesser concern. This will be discussed in detail later in the article. For now, it is important to understand that simply carrying a mutation does not necessarily mean an individual will get cancer - it only means that his or her risk is elevated. The practical implication of this statement is that we will probably never eliminate cancer completely from our population (human or canine), but we can achieve a significant reduction in the number of cases as we learn more about how the disease happens and we work to diminish risk factors or detect the disease early enough to treat it before it becomes a life-threatening condition.

The news is not all bad. We have identified some of the fundamental properties of canine hemangiosarcoma, and it is possible one or more of these may prove to be an "Achilles heel" for the tumor. For example, most of these tumors make growth factors that they need to survive, or they "coerce" cells in their environment to do this for them. One of these growth factors is vascular endothelial growth factor-A or VEGF, which acts by binding specific receptors on the hemangiosarcoma cells. New drugs under development by various pharmaceutical companies are designed specifically to interfere with the signals transmitted by these receptors. The reliance of hemangiosarcoma cells on VEGF signals to survive should make them more sensitive than normal cells to these drugs. Several groups are working to bring these drugs into the clinic, but the process is slow because testing must be done in a careful, deliberate way to ensure the compounds are safe and effective. The rules for participation in clinical trials are stringent, but if you have a dog that is diagnosed with terminal hemangiosarcoma, your veterinarian may have information about trials for which he or she may be eligible. As we will detail below, our work and that of others continues to illuminate new avenues that we may be able to use to more effectively prevent, control, and treat this disease.

Treatment for Canine Hemangiosarcoma

Regrettably, the standard-of-care for this disease has not seen significant advancement over the past 20 or 30 years. There is presently no readily available, effective test for early diagnosis of hemangiosarcoma. Careful analysis of blood samples by experienced pathologists may hint at the presence of chronic hemorrhage and blood vessel abnormalities that are suggestive of hemangiosarcoma. However, this method is neither sensitive nor specific to confirm the diagnosis. Non-invasive imaging methods are useful aids to diagnose the disease. In particular, ultrasound is moderately specific, but it is not sensitive, and the tumor must be large enough to be grossly visible. In addition, biopsies are required for confirmation of imaging results. Repeated biopsies of tissues where the tumors may arise (without other evidence for the presence of a tumor) are of little use to provide early diagnosis, and considering the fact that there is some risk to these procedures, such an approach is practically and ethically unacceptable.

The options for therapy of canine hemangiosarcoma are limited, largely because the disease is not diagnosed until the late stages. The standard consists of surgery to shrink or remove the primary tumor, when possible, followed by intensive chemotherapy. In some cases, surgery is not feasible, or it can be impractical or inappropriate (for example, if there is evidence of extensive metastatic spread to sites beyond the primary tumor). Median survival for dogs treated with surgery alone is approximately 90 days, and that is extended to approximately 180 days by the addition of chemotherapy using one of several protocols available. Because the goal for chemotherapy in pet dogs is to extend life with good quality, toxicity is generally not a major issue of concern, and when it occurs it is most often managed without much difficulty.

There is no other therapy that has been proven to be effective to manage or control hemangiosarcoma. As we will mention below, the cells that give rise to this disease originate in the bone marrow. We do not yet know when (or why) they localize to the organ where the disease will arise, but this means that prophylactic splenectomy (removing the spleen to prevent the development of the disease) is unlikely to have any benefit, since in the absence of a spleen, the transformed cell (or cells) can simply go to another organ. Also, the spleen is not simply a "window dressing" organ,

and even though dogs can function without a spleen, they will have reduced ability to adapt or respond to a variety of conditions.

Several alternative and complementary approaches (diet, herbs, mystical energy, etc.) have recently become popular as people try to find treatments for canine hemangiosarcoma. This usually follows extensive publicity (such as from Internet chat groups) after a dog receives these treatments and survives longer than anticipated, leading proponents to advertise this as success and evidence that their approach is curative for hemangiosarcoma. The danger of attributing curative power to treatment approaches after an anecdotal response cannot be overstated. There is no reported case where one of these therapies has been consistently successful (or even as good as the standard of care) after it has been tested without bias to try to replicate the anecdotal response. In fact, sometimes such treatments can actually interfere with - or increase the toxicity of chemotherapy drugs. We know that when some tumors are caught early enough, surgery alone, surgery plus chemotherapy, and in some cases no treatment at all can lead to extended survival. In rare instances, the behavior of the tumor itself is such that disease progression is extremely slow and dogs can survive for an extended period regardless of the therapy used. This means that a small proportion of dogs diagnosed with hemangiosarcoma will live 2, 3, 4 years and longer even without aggressive management. Therefore, it is essential for families who have dogs that are diagnosed with cancer to recognize two things. One is that veterinarians would not willingly hold back effective therapies for any disease. Our job and our responsibility are to improve the health and well being of dogs and their families, and all of us would welcome an effective and non-toxic cure for this disease. The other is that there is no shortage of predators on the Internet and elsewhere who benefit from the desperation people feel when they know a beloved family member will probably die from a serious, incurable disease.

Hope for the Future

The unwavering support of the dog-loving community has already made a difference in our understanding of canine hemangiosarcoma. To end this article, we will highlight new information that makes us optimistic about our chances to win the battle against this disease. Here, we wish to note our gratitude for the support that our group and our collaborators have collectively received from dog clubs and owners alike, both financial and by making samples available for ongoing studies.

Development of a Test for Early Detection. As we mentioned above, one way to improve our success treating hemangiosarcoma is by detecting the disease early. Previous work from our group showed there are unique patterns of protein expression that can help distinguish hemangiosarcoma cells from normal blood vessel lining cells, even within the tumor. We also knew that most tumors shed some cells into the circulation. We reasoned, then, that we could take advantage of this to develop a test to look for tumor cells in the circulation, providing a minimally invasive, accurate diagnostic test for hemangiosarcoma. In many ways, this is similar to the proverbial search for a needle in a haystack. The tumor cells are so infrequent that they cannot be detected in routine blood tests, nor are they easy to enrich. To accomplish our goal, we needed to use a robust technology called flow cytometry. For this, cells are "tagged" with fluorescent molecules that identify their lineage of origin and then cells are run through an instrument (the flow cytometer) that combines sophisticated optics, fluidics and software that make it capable of analyzing tens of thousands or hundreds of thousands of cells in a matter of minutes. With financial support from the Portuguese Water Dog Foundation, Inc., the Portuguese Water Dog Club of America, and Idexx Laboratories, we were able to show proof of principle for this test. The work was published in the July 2006 issue of the journal *Experimental Hematology*. Idexx Laboratories has licensed the technology from the University of Colorado. As of the writing of this article, the test is in the final stages of optimization and transfer to Idexx for final testing. Several questions remain to be answered before the test can be offered commercially, not the least of which are cost analysis and market research to ensure that the final product is not only diagnostically useful, but also affordable for veterinarians and pet owners.

A planned future use for this test is its application to detect the presence of hemangiosarcoma in dogs at risk before the tumor poses a clinical hazard. As is true for other tumors, early detection is likely to offer the highest probability of successful treatment outcomes. There is, however, an important caveat. The presence of hemangiosarcoma cells in the circulation does not tell us where in the body the tumor is likely to develop, as tumors in the spleen, liver, heart, and skin all produce positive results in this test. Therefore, we have initiated several studies with support from the AKC Canine Health Foundation, the National Canine Cancer Foundation, and others, to develop novel treatment strategies for hemangiosarcoma that are independent of the site of origin (these are still in the earliest stages of laboratory development). Only then will we be able to ethically and judiciously use early detection to improve outcomes for dogs affected with this disease.

Is Hemangiosarcoma a Disease of Stem Cells? A lot of attention has been focused recently on stem cells and their potential use to cure a wide variety of diseases. The "dark side" of stem cells is that these may be the only cells that can

give rise to tumors. It is very important to note here that this is not a reason to avoid or abandon research on the therapeutic promise of stem cells, as there is no practical, documented evidence that the possibility to develop cancer is any higher in laboratory animals or patients that receive stem cell transplants.

Until recently, the dominant theory for the origin of cancer assumed that all cells possess an equal capacity for self-renewal; that is, the capacity to make a new identical cell that retains all the properties of the mother cell, and that proliferation of cancer cells was a random process driven entirely by selection of mutations that increased the fitness of a cell in a particular environment. A competing theory now exists whose main concept is that self-renewal is limited to a small population of "cancer stem cells." These cells possess the two features that define "stemness": (1) self-renewal and (2) multipotency (the capacity to make new cells that can differentiate into more than one type or lineage). In other words, a very small number of self-renewing cells in a tumor generate large numbers of progeny that make up the bulk of the tumor (or tumors). The existence of "cancer stem cells" is now well documented; they have unique properties of gene and protein expression, and the initial transformation seems to involve a relatively small number of mutations. The accumulation of additional mutations determines the extent to which the progeny from these cells will differentiate, variably resulting in distinct clinical entities such as high-grade, aggressive tumors and low-grade, indolent tumors.

This model of cancer explains why multicellular animals like a dog, with trillions of cells in their bodies, can survive to reproductive age without developing cancer, as the capacity for tumor formation is limited to perhaps a few hundred "stem cells", dramatically reducing the probability that any cell with cancer-causing potential will accumulate the set of mutations needed to develop the disease. Still, the high incidence of cancer among older individuals reinforces the notion that even with a limited number of susceptible stem cells, the probability to develop cancer over a long life is quite high. With regard to canine hemangiosarcoma, recent data from our lab support the idea that this is a disease of stem cells, the first such evidence for a canine tumor. Current work is focused on characterizing these stem cells, as their unique properties may provide opportunities to develop highly targeted, non-toxic therapies to effectively treat this disease.

What Are the Breed-Specific Risk Factors for Canine Hemangiosarcoma? We mentioned previously that hemangiosarcoma is a rather common cancer in certain breeds, including Golden Retrievers, German Shepherd Dogs, and Portuguese Water Dogs. This tells us that heritability contributes to risk, and so it may be possible to reduce the incidence by eliminating the highest risk alleles from the population. Moreover, if risk factors responsible for the disease are unique among breeds, we might have to consider the possibility that various new therapy approaches will have to be developed to effectively treat this disease in different breeds of dogs. To date, we have taken several approaches to identify how heritable factors influence hemangiosarcoma risk. One approach, developed largely in collaboration with Dr. Elaine Ostrander at the National Human Genome Research Institute of the National Institutes of Health and Dr. Kerstin Lindblad-Toh at the Broad Institute of MIT and Harvard, seeks to map risk alleles in Portuguese Water Dogs, Golden Retrievers, and German Shepherds using resources made available by the recent completion of the Canine Genome Project. We continue to collect samples for these projects, and we would be extremely grateful to owners who wished to participate by contributing blood and pedigree information from healthy (aged) dogs and from affected dogs. Information for participation is available at http://www.modiolab.org/studyInfo/studyInfo_index.shtml. A second approach, developed largely in collaboration with Dr. Matthew Breen, seeks to determine breed specific abnormalities that are detectable in tumors obtained from dogs of different breeds. We have made significant progress on this project, and have new data showing that, indeed, tumors from dogs of one specific breed are functionally and genetically more similar to each other than they are to tumors from dogs of other breeds. We are extremely excited about this information, as it will, for the first time, provide tangible evidence that heritable risk factors are a contributor to the development of canine cancer. More importantly, these results are the first step in our long-term goal to develop strategies for prevention and treatment that address specific causes underlying the biology of canine hemangiosarcoma.

In conclusion, hemangiosarcoma remains a devastating, untreatable disease of dogs. However, ongoing work in our laboratories and elsewhere is rapidly increasing our understanding of this disease, providing hope that we will achieve our goals to reduce its incidence and control its impact on dogs and their human families within our lifetime.

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