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A Compilation of Homologous Cancer-Related Genes in Humans and Dogs

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**A COMPILATION OF HOMOLOGOUS
CANCER-RELATED GENES IN HUMANS AND DOGS**

A Thesis Submitted
in Partial Fulfillment
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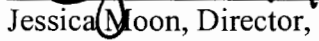
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Introduction

Cancer is a disease commonly found in many species, including humans and domestic dogs. Cancers vary greatly in their appearance, behavior, and prognosis, but there is one element that all cancers share. All cancers have some type of mutation that alters gene expression (Klug et al., 2000). Most of these mutations are not passed on in the germ-cell line; in fact, only about 1% are passed on. However, when mutations do get passed on to offspring, they can cause the offspring to become more susceptible to developing cancer at some point in their life.

Mutations that increase cancer susceptibility can be several different types, including single nucleotide substitutions, chromosome rearrangement, chromosome gain or loss, or integrated viral genes (Klug et al., 2000). If offspring inherit one mutant allele, their predisposition to the development of cancer can increase significantly, even if they do have one normal allele of the same gene. Genes that are known to increase the risk of developing cancer when mutated are known as cancer-susceptibility genes. Alleles in these genes may play a role in both familial and sporadic cancer.

Cancer cells are identified by two main characteristics: uncontrolled growth and the potential ability to spread to other sites in the body (metastasis) (Klug et al., 2000). These cells, for some reason, have lost control over the cell cycle, which allows the cells to proliferate very rapidly. The cell cycle is normally regulated at the transition between the G1 and S phases, and also at the transition between the G2 and M phases. At these two points, a decision to proceed or to halt must be made. This decision is controlled by the interaction of two classes of proteins: protein kinases and cyclins. Protein kinases are enzymes that selectively phosphorylate target proteins. Cyclins are proteins that control

progression through the cell cycle (Klug et al., 2000). When protein kinases and cyclins interact, a regulatory molecule is produced, controlling the movement of the cell through its cycle. Therefore, any mutations that disrupt steps of the cell cycle can be cancer-causing, especially mutations in genes that code for cyclins or protein kinases (Klug et al., 2000).

Mitosis in cells is regulated by two different types of genes, tumor-suppressor genes and proto-oncogenes. Tumor-suppressor genes suppress cell division, so therefore their genes and the proteins they code for must either be absent or inactive for cell division to occur (Klug et al., 2000). If tumor-suppressor genes become permanently inactivated or lost because of mutation, control of the cell cycle is lost and cells begin to divide uncontrollably (Klug et al., 2000).

The second type of cell regulator, proto-oncogenes, promote cell division. To stop cell division, these genes must be inactivated. If they become permanently switched on, uncontrolled cell division results, leading to tumor formation (Klug et al., 2000).

Mutated proto-oncogenes are generally referred to as oncogenes.

Generally, cancer is caused by several mutations. It usually takes a mutation in at least one tumor-suppressor gene and at least one proto-oncogene. It is possible that a tumor will develop because of only one mutation, but the likelihood that it is malignant increases if more mutations are present.

Dogs as a Model

The domestic dog would provide an excellent model for research on cancer leading to advances in human medicine for several reasons. Cancer is fairly prevalent in

dogs; in fact, one study found that 45% of dogs in the United States over the age of ten died of cancer, as well as 23% of dogs of any age (Olson et al., 2004). Dogs get various types of cancer like humans do, with greater frequencies in certain breeds, suggesting a genetic influence. Dogs would be a particularly useful model in human research because they share a common environment with their owners. Genetic problems are only one risk factor for developing cancer, so it is helpful to consider other factors, such as environment and nutrition. Dogs provide a great opportunity to look at the environmental factors as well as genetic factors. Another benefit to using dogs as a model is that the latency period for developing cancer is shorter in dogs than humans, making it easier to get results in a shorter period of time (Olson et al., 2004).

Dogs also provide a unique model for study because there are extensive records of their lineages. Due to registration requirements for purebred dogs, it is possible to trace genetic lineages for many generations, while information is limited to a few generations in many human cases (Olson et al., 2004). In addition, purebreds can provide a great deal of helpful information, since they are essentially 300 partially inbred genetic isolates. The amount of restriction that has been imposed on gene flow between breeds allows for the study of many genetic problems (Olson et al., 2004). Inbreeding is often practiced in the selection of mates for purebred dogs. Brother/sister or parent/offspring pairings are generally avoided, but slightly less related dogs are often chosen to breed (Ostrander et al., 2000). This causes a much greater incidence of expression of recessively inherited disease traits. This unintended effect can cause enormous problems for breeders and owners, but it leads to amazing opportunities for genetic studies (Ostrander et al., 2000).

Many canine disorders, including cancer, do not appear until later in a dog's life. In purebreds, this can mean that the dog has already been bred and has passed on the genetic predisposition to develop cancer (Olson et al., 2004). If studies could be done that would reduce genetic disease in dogs, it would benefit dog owners and breeders, as well as provide potentially valuable data for human research. It would save a significant amount of money each year in producing purebreds and guide dogs (Olson et al., 2004). The Whitehead Institute and MIT Center for Genome Research are currently working together to sequence the entire genome of a Boxer. They are also working on sequencing 100,000 short random genome segments from ten other breeds. They plan to compare these segments to the Boxer genome in order to identify differences that could be used as DNA markers (Olson et al., 2004). They believe that there should be a marker in or near each gene in the canine genome, which contains about 40,000 genes.

Dogs seem to get cancer about twice as frequently as humans, but the presentation, histology, and biology of several canine cancers closely resembles human cancers (Ostrander et al., 2000). Some of the cancers that are the most similar between the two species are osteosarcomas, mammary carcinomas, oral melanomas, lung carcinomas, and malignant non-Hodgkins lymphomas (Ostrander et al., 2000).

The most commonly detected tumors in both dogs and humans are found in either the skin or the mammary glands (Richards et al., 2001). This may be due to the fact that these areas are among the easiest to detect abnormalities in, and also because they are more likely to be excised and examined for cancer than are tumors of internal organs. One third of all tumors detected in dogs are found in the skin or in subcutaneous tissues (Reimann et al., 1999).

Dogs would also make a good model for human cancer research because the degree of medical attention given to dogs is second only to humans (Ostrander et al., 2000). The heightened amount of care they receive makes them attractive for cancer studies because many dogs would be available to gather data from (Richards et al., 2001).

Common Cancers in Dogs

Breast cancer is the most common cancer in both female dogs and female humans. While 97% of all breast cancer is found in female dogs, males do get breast cancer on rare occasions (Bessinger, 1998). Out of all canine mammary tumors, 76% are adenocarcinomas, which are the most prevalent mammary tumors in humans as well. Spaying dogs at a young age can help protect against breast cancer, but that is obviously not an option in humans. Purebred dogs are twice as likely as mixed breeds to get breast cancer, possibly due to the fact that they are spayed much less often (Bessinger, 1998).

While male dogs are not likely to get breast cancer, they do develop testicular cancer. Humans tend to get mainly one type of testicular cancer, seminomas, but dogs are susceptible to Sertoli cell tumors and interstitial cell tumors, as well as seminomas (Bessinger, 1998). Dogs with undescended testicles are at the highest risk for developing testicular cancer. The risk for testicular cancer development can be reduced by neutering.

Dogs also get lymphoma (cancer of the immune system cells) quite often. The type of lymphoma they develop is very similar to non-Hodgkin type lymphoma in humans (Bessinger, 1998). Male and female dogs are affected at the same rate, and neutering or spaying does not appear to have any effect on the development of lymphomas. Interestingly, lymphoma development in dogs has been associated with high

exposure to electromagnetic fields (Bessinger, 1998). It is possible that future research on canine lymphomas could address concerns about human exposure to electromagnetic fields.

Osteosarcomas, or bone tumors, are very prevalent in dogs as well as humans. Tumors in the metaphysis, or site of bone growth, are the most common osteosarcomas in both species. These tumors tend to be high grade, aggressive tumors that spread easily, often to the lung (Bessinger, 1998). Males are more likely to develop bone cancer than females, and spayed and neutered dogs have more than twice the risk of intact dogs, suggesting that hormones may play a role. Bigger dogs also develop osteosarcomas at a much higher rate than small dogs, with dogs over 80 pounds 61 times more likely to get bone cancer than smaller dogs (Bessinger, 1998). It is also thought that strenuous activity may cause microscopic fractures in bones during rapid periods of growth, which could induce cancer formation (Bessinger, 1998).

Another type of cancer that dogs are prone to developing is bladder cancer. Bladder cancer is more common in older dogs, and is also more common in dogs that are given flea or tick dips or are obese (Bessinger, 1998). However, it is important to keep in mind that these factors may just be association factors, not necessarily a cause of bladder cancer. Female dogs are more likely than male dogs to develop bladder cancer, possibly because they urinate less often and have more body fat, which stores chemicals, making them more susceptible to developing cancer (Bessinger, 1998).

Nose cancer is fairly common in canines, and the tendency to develop this type of malignancy increases with age. Long-nosed breeds are much more likely than short-nosed breeds to be susceptible to nose cancer, possibly because long-nosed dogs more

effectively filter carcinogens with their nasal lining (Bessinger, 1998). This would prevent the carcinogens from entering the rest of the body, but would cause them to come into greater contact with the lining of the nose itself, which could induce cancer formation. In addition to nose length, the frequency at which dogs develop nose cancer almost doubles when they are living in a home with a smoker (Bessinger, 1998). This correlation could be useful in future studies on the effects of secondhand smoke on humans.

Dogs are the only non-human species that suffer from a significant amount of prostate cancer. However, unlike humans, canine prostate cancer is extremely aggressive and spreads quickly to lymph nodes, the lungs, and bones (Bessinger, 1998).

Lung cancer is prevalent among humans. It is the second most common cancer in both men and women, but is not often found in dogs. Dogs do spontaneously develop lung cancer; however, less than 1% of dogs develop this type of cancer (Bessinger, 1998). This is probably due to the fact that most human lung cancer is caused by smoking, which is not a risk factor for dogs.

The Dog Genome

The dog has a diploid genome, made up of 78 chromosomes (Switonski et al., 2004). It is smaller than the human genome due to the fact that it contains a lower percentage of repetitive sequences. However, genetic diseases in dogs are often caused by a mutation in the same gene as their human counterparts. Similarly, dogs, as well as humans, are susceptible to developing both familial and sporadic cancers (Switonski et al., 2004). Some canine malignancies are very similar to human cancers and show almost

identical responses to therapy. This suggests that if gene therapies were developed to treat cancer in dogs, it would be possible to apply this knowledge toward human cancer research and further the possibility of developing human gene therapy. Unfortunately, studies that have been done on dog cancer are not as advanced as investigations into human cancer, simply because it has been difficult to identify all dog chromosomes with classical banding techniques used with human DNA (Switonski et al., 2004).

The dog genome is especially interesting for cancer research because different breeds of dogs are at a greater risk of developing certain cancers, possibly indicating that there are specific genetic mutations involved in various malignancies. For example, Pointers, Poodles, Cocker Spaniels, and Boston Terriers are at a higher risk of developing mammary cancer than are other breeds (Ostrander et al., 2000). Osteosarcomas are more common in larger breeds, such as Great Danes, St. Bernards, Doberman Pinschers, and Labrador Retrievers (Ostrander et al., 2000). It is possible that these cancers have a higher risk in certain breeds due to environmental factors. For example, the breeds at an increased risk for bone cancer are all large dogs, so it is possible that their increased risk comes from the increased stress their bones are placed under. However, it is important to consider the possible genetic influences. Mapping the cause of cancer within breed-specific cases could provide a good opportunity to learn more about the genetic basis of cancer. Canine pedigrees could be used to look for the genetic mutations that caused the predisposition to develop such a breed-specific disease (Switonski et al., 2004).

Proto-oncogenes

K-ras

There are several proto-oncogenes that have been found to be homologous between humans and dogs. One of the most studied of these genes has been k-ras. K-ras normally works with other genes of the ras family to produce a 189-amino acid protein in cell membranes that is involved in the transduction of signals across the cell's plasma membrane (Klug, 2000). Substitution of just one amino acid can cause this gene to become permanently switched on, leading to tumor formation. This gene has been linked to both lung cancer and pancreatic adenocarcinoma. Griffey et al. (1998) examined the relationship between k-ras mutation and lung cancer, which is the most common cause of death for both men and women in the United States, with the majority of these deaths linked to cigarette smoking. It has been found that the most common genes altered in human lung cancer are from the ras family of oncogenes, especially k-ras, which is mutated in up to 30% of all lung cancers (Griffey et al., 1998). The dog may be a useful model for the study of pulmonary carcinogenesis, because humans and dogs share a highly homologous k-ras gene. However, less than one percent of all dogs develop lung cancer, which is vastly less than the human rate. Griffey et al (1998) examined 126 spontaneously arising canine lung tumors for mutations in exons 1 and 2. Of the tumors used in the experiment, there were 9 non-cancerous adenomas, 59 bronchioalveolar carcinomas, 30 adenocarcinomas, 16 adenosquamous carcinomas, 3 squamous cell carcinomas, and 9 anaplastic carcinomas. Of the tumors examined, k-ras mutations were found in 19 of them. One mutation was found in exon 2 codon 61, and the other 18 mutations were found in exon 1 codon 12. Therefore, the frequency of the k-ras

mutations found in malignant tumors was 16.2% (Griffey et al., 1998). There were no mutations found in the non-cancerous tumors considered.

The most common mutation found was a G→ A transition at the second position of codon 12, which made up 59% of the k-ras mutations discovered. The second most common mutation found was a G→ T transversion at the second position of codon 12, which made up 26% of the k-ras mutations discovered (Griffey et al., 1998). In humans, tumors from non-smokers have a 100% rate of G→ A transitions at the second position of codon 12, which suggests that dogs and humans that have this mutation may acquire it from carcinogens that are not found in tobacco smoke (Griffey et al., 1998). People that have been found to have this particular mutation have also had a better prognosis than those with other k-ras mutations (Griffey et al., 1998). Interestingly, 60% of the canine lung tumors with k-ras mutations showed evidence of metastasis, while only 32% of the tumors without k-ras mutations metastasized (Griffey et al., 1998). This could mean that k-ras mutations play a significant role in whether or not lung tumors spread to other tissues.

The frequency of k-ras mutation in humans who smoke has been found to be 30-32%, while the frequency in non-smokers is only around 7%. In adenocarcinomas (the most common human lung cancer), dogs have a k-ras mutation rate of 11%, which is most similar to that of non-smokers (Griffey et al., 1998). This suggests that there are probably multiple causes for the spontaneous development of lung cancer in dogs, one of which may be secondhand smoke. The fairly low frequency of k-ras mutations in canine lung cancer also suggests that many lung tumors in dogs may have genetic abnormalities that have yet to be discovered.

In a different study done by Griffey et al. (1998) at the Inhalation Toxicology Research Institute on the rate of k-ras mutation in canine lung cancer, the effects of direct radon exposure on the lungs were analyzed. Inhaled radon has been known to cause lung cancer in humans, especially in miners who are exposed to very high radon content. Radon emits alpha particles, and it has been determined that the inhalation of plutonium (^{239}Pu) alpha particles can cause a large amount of energy to build up in the airways of the lung, leading to cancer (Griffey et al., 1998). In previous studies done by Griffey et al. (1998), it had been determined that the inhalation of plutonium by beagles often caused them to develop lung cancer. This study went one step further by looking for k-ras mutations in these lung tumors.

Eighteen-month old beagles were given a single dose of inhaled $^{239}\text{PuO}_2$ and allowed to live out the rest of their natural lifespan in the laboratory. After death, tumors were removed from 25 dogs and examined for k-ras mutations. Two out of the 25 tumors had mutations in exon 1 of k-ras, and both were GGT→GAT transitions at codon 12 (Griffey et al., 1998). The tumors only had a k-ras mutation rate of 8%, which is less than spontaneously occurring lung cancer in dogs (16%) and spontaneously occurring mutations in human lung cancer (13-36%). However, the two mutations found were similar to the most frequent mutation in spontaneous canine lung tumors (Griffey et al., 1998). This suggests that it is possible that the two mutations in this study occurred independently from the exposure to $^{239}\text{PuO}_2$, and were caused by carcinogens in the environment. These results indicate that inhaling radon does not cause k-ras mutations in dogs. If the same results could be obtained in studies of humans, it could allay fears about radon gas exposure in homes.

Mayr et al. (2003) examined the rate of k-ras mutation in pancreatic adenocarcinomas. Pancreatic adenocarcinomas are not as common as are other cancers in dogs, but dogs have a higher incidence of these types of cancer than do any other species (Mayr et al., 2003). The majority of these adenocarcinomas are ductal and are very difficult to diagnose while in the early stages. These tumors are usually in the advanced stages when they are finally diagnosed, and generally have a poor prognosis and short survival time.

The k-ras proto-oncogene, when mutated, may cause pancreatic tumors to form by causing the permanent activation of growth-related signal transduction pathways (Mayr et al., 2003). The mutations that are the most common in these tumors in humans are usually found in codons 12, 13, and 61. These codons are mutated in around 15-20% of human malignancies, often in pancreatic, colorectal, and lung cancer (Mayr et al., 2003). So far, very few mutations have been found in the dog k-ras gene in malignant tumors. This experiment, however, did find a good deal of k-ras mutation in canine pancreatic carcinomas.

The researchers examined tissues from five dogs between the ages of six and twelve that had suffered from pancreatic carcinoma. Four out of the five tumors examined had a point mutation in codon 12 of exon 1 of the k-ras gene (Mayr et al., 2003). Three of these mutations were GGT→GAT transitions and one dog had a GGT→CGT mutation. The GGT→GAT mutations are consistent with the most common k-ras mutation in previous studies.

In humans, the highest incidence of k-ras mutation occurs in ductal adenocarcinomas of the pancreas, as 70-100% of these tumors show a mutation in this

gene (Mayr et al., 2003). Given that 80% of the canine tumors in this study also exhibited k-ras mutations, it appears that codon 12 of the k-ras exon 1 could be a very important mutation site for pancreatic adenocarcinomas. Dogs and humans share a great deal of homology in this gene, so dogs would be excellent models for research on human pancreatic cancer. The need for research in this area is great, considering that survival rates for humans with pancreatic cancer has hardly changed in several decades (Mayr et al., 2003).

C-kit

Another proto-oncogene affecting both dogs and humans is the c-kit proto-oncogene. The c-kit gene encodes the KIT cell surface receptor. When KIT is activated, it binds and phosphorylates a class of intracellular substrate proteins, initiating a signaling cascade (Ma et al., 1999). This cascade causes mast cells to proliferate, migrate, and mature. KIT can be activated by a mutation in its surface receptor, caused by a c-kit mutation, and once activated can become oncogenic.

Having an increased number of mast cells in various organs produces a condition known as mastocytosis, and the neoplasms that are produced are called mastocytomas (Ma et al., 1999). Mastocytomas make up 7-21% of all canine tumors, and are often aggressive and metastasize to other tissues, including the lymph nodes, liver, spleen, and bone marrow (London et al., 1999). In contrast, human mastocytomas almost never spread to other tissues. In previous studies on human c-kit, several mutations have been found in mast cell lines that cause the activation of KIT.

Ma et al. (1999) studied c-kit DNA from six mastocytoma tissues and cell lines. Of these mastocytomas, five contained mutations in their juxtamembrane region that

caused the activation of KIT (Ma et al., 1999). This suggests that the juxtamembrane region may be a key region involved in the development of mast cell neoplasias.

Canine KIT is 88% homologous with human KIT and 97% homologous in the intracellular domain (Ma et al., 1999). To test the functional significance of the juxtamembrane region mutations, the experimenters created an equivalent mutation in human tissue and grew the sample in a dish. The mutations did appear to cause the activation of KIT, but it was not clear whether or not the c-kit mutation alone was enough to induce the formation of mast cell neoplasias (Ma et al., 1999).

London et al. (1999) searched for specific c-kit mutations in canine cancers. Previous studies had shown that spontaneously arising point mutations in the kinase domain of human c-kit had been found in three mastocytoma lines (P815, RBL, and HMC-1) (London et al., 1999). These point mutations appear to cause phosphorylation of KIT and may aid the progression or development of mast cell tumors. KIT is a transmembrane tyrosine kinase receptor and is also related to receptors for growth and stimulating factors (London et al., 1999). The binding of KIT appears to promote the development, survival, and differentiation of mast cells.

The purpose of this study was to find out if spontaneously arising point mutations occurred in canine c-kit and if these mutations contributed to the development of mast cell tumors in dogs. Specimens were collected from 11 dogs after tumors had been surgically removed or the dog had died. The c-kit DNA was amplified using PCR, and the previously established point mutations were searched for (London et al., 1999). The point mutations found in human mast cell lines were not detected in the canine c-kit DNA, but new mutations were observed. These new mutations, observed in 50% of the

tumors, were tandem duplications in exons 11 and 12 of c-kit (London et al., 1999). Each DNA sample had a unique 45-70 base pair duplication at the 3' end of exon 11, which caused an aberrant product to be produced (London et al., 1999). These mutations provide evidence that c-kit may play a role in the development of spontaneous canine mast cell tumors, but their specific effect remains unknown.

The human mast cell tumors with point mutations in c-kit differed from the canine mast cell tumors with the tandem duplications in a few ways. In humans, the point mutations contributed to systemic mastocytosis, which is where the mast cells are well differentiated but can still infiltrate multiple organs (London et al., 1999). In dogs with tandem duplications, the mast cell tumors were poorly differentiated. They began as solid tumors that only spread to local lymph nodes (London et al., 1999). The biology of the two mast cell tumors is clearly different.

Although the tumors themselves differ between the two species, the c-kit gene is very similar. Within exons 11 and 12 of the c-kit gene, there is 100% homology between humans and dogs (London et al., 1999). The duplication mutations found in the dogs were all slightly different, but they were also all found in the 3' region of exon 11. Exon 11 encodes the juxtamembrane domain, which is believed to participate in the negative regulation of KIT (London et al., 1999). When this area is mutated, KIT becomes overactive. Since all the mutations in the dogs and a great deal of human c-kit mutations are found in this region, it is possible that the juxtamembrane region has some inherent genetic instability (London et al., 1999). It appears that any KIT alterations can lead to the proliferation of mast cell tumors, regardless of the type of mutation that causes the

alterations. Future research into mutations of the juxtamembrane region of canine c-kit could provide helpful information towards the research on human c-kit.

C-yes

The c-yes oncogene is common in many human tumors, and it is believed to play an important role in tumors of dogs as well. Normally functioning c-yes is involved in cell growth of both adult and fetal tissue. Rungsipat et al. (1999) studied the role of c-yes in 185 spontaneously arising canine tumors of various types. These tumors were tested for the c-yes oncogene using an antibody for human c-yes, and some of these tumors tested positive for the gene. This indicates that the c-yes oncogene could play a role in cell growth and metabolism in these cancerous cells. Of the tumors examined, the number that tested positive for c-yes varied considerably by tumor type. Overall, 59% of the tumors exhibited the c-yes oncogene product. Of these, 44% of skin tumors, 5.5% of round cell tumors, 35.7% of soft tissue tumors, 21.4% of testicular tumors, 29.1% of mammary tumors, and 52.6% of other tumors expressed the c-yes oncogene product (Rungsipat et al., 1999). This degree of variation in expression is common in spontaneously arising tumors. There has not been a great deal of research done into the similarity between human c-yes and canine c-yes, so future research into this area would definitely be worthy of interest.

HER-2

HER-2, also called c-erbB-2, is a proto-oncogene that normally encodes a tyrosine kinase receptor protein (Mulas et al., 2003). In humans, overexpression of HER-2 is correlated with aggressive tumor behavior and drug resistance in breast cancer. HER-2 protein is overexpressed in 15-30% of human breast cancer cases, and 85-90% of

these tumors have amplification of the HER-2 gene (Mulas et al., 2003). Mammary gland carcinomas in dogs have similar epidemiological, clinical, morphologic, and prognostic features to human breast cancer (Mulas et al., 2003). Studies have shown that either the HER-2 gene is amplified in canine mammary tumors, or that the HER-2 protein is overexpressed, but no studies had shown that both conditions exist at the same time in dogs.

Mulas et al. (2003) examined 23 canine mammary lesions for both gene amplification and protein overexpression. Of the 23 lesions, 17 were invasive carcinomas, and 6 were benign lesions used as a control (Mulas et al., 2003). HER-2 protein overexpression was found in 3 out of 17 carcinomas and zero of the benign lesions. The carcinomas with HER-2 protein overexpression were made up of atypical epithelial cells, had a high histologic grade of malignancy and infiltrative growth, and also lacked estrogen and progesterone receptors (Mulas et al., 2003). None of the dogs with these tumors had received therapy, and all had either suffered from metastasis or died within six months of surgery. Around 17.6% of the malignant tumors showed overexpression of HER-2 protein, which is similar to the rate in human breast cancer (Mulas et al., 2003). Human tumors with overexpressed HER-2 protein also show poor prognosis, high histologic grade of malignancy, and the absence of steroid hormone receptors similar to dogs.

Surprisingly, there was no difference in the number of HER-2 gene copies between cases with and without HER-2 protein overexpression. In humans, this gene is amplified at a very high rate in breast cancer, and dogs were expected to follow this pattern. However, 3-15% of human breast cancer cases do show HER-2 protein

overexpression without HER-2 gene amplification (Mulas et al., 2003). A canine model would be suitable to study for this small subset of human breast cancer, and further research of the HER-2 protein could also be highly valuable in the search for breast cancer treatment.

HMGA1

The human HMGA1 gene codes for three proteins through alternative splicing- HMGA1a, HMGA1b, and HMGA1c (Escobar et al., 2004). The HMGA1 proteins are normally abundantly expressed in embryonic cells and play a role in cell division and growth. The HMGA1a and HMGA1b proteins have also been associated with various human diseases, including cancer. These proteins are able to modify chromatin structure by bending DNA, and therefore influence the transcription of several target genes (Escobar et al., 2004). In humans, the HMGA1 gene is located on chromosome 6p21, which is often affected by mutations. Higher levels of HMGA1 have been associated with colorectal, cervical, pancreatic, prostate, and thyroid cancer (Escobar et al., 2004). It appears that the more HMGA1 protein that is present, the more aggressive the tumor's behavior is. Experiments have been done that show that reducing the amount of HMGA1 can significantly reduce the size of tumors (Escobar et al., 2004).

The canine HMGA1 gene is located on chromosome CFA23. It contains 6 exons and codes for HMGA1a and HMGA1b proteins, as it does in humans. The HMGA1 genes are 80.6% homologous between humans and canines, and the HMGA1a and HMGA1b proteins are 100% homologous (Escobar et al., 2004). Mice and other research animals have not been found to be nearly as homologous with humans. The identical structure of human and dog HMGA1 proteins could mean that therapy developed using

dogs would easily transfer to humans. So far, the amount of HMGA1 expression in canine tumors has not yet been studied. If high levels of HMGA1 in canine tumors were found to be related to aggressive tumor behavior and large tumor size, research into this gene could provide a significant impact on many types of cancer.

Tumor-Suppressor Genes

p53

The second type of cell cycle-regulating genes that can be involved in cancer development are tumor-suppressor genes. Of the tumor-suppressors, p53 is the most common gene involved in cancer. The p53 gene encodes a nuclear protein that acts as a transcription factor. It controls the passage from the G1 to S phase in the cell cycle (Klug et al., 2000). An inherited mutation in this gene could cause a predisposition to developing several types of cancers in different tissues.

Cells normally have a low level of p53, but the amount increases a great deal when the cell's DNA is damaged. When damage occurs, p53 exhibits one of two responses. It either causes the cell cycle to stop and the DNA to be repaired, or it causes apoptosis (Klug et al., 2000). If cells do not have functional p53 protein, they are able to progress throughout their cycle with damaged DNA, which could mutate even further. Since p53 protects cells from progressing with mutations, it is sometimes known as the "guardian of the genome" (Klug et al., 2000).

Over 60% of all human tumors contain a p53 mutation (Mayr et al., 1997). Of these tumors, 85.6% are missense mutations, and 92.1% occur between codons 120 and 290. This region is highly conserved between species, and contains six mutation "hotspots" (Mayr et al., 1997). These spots are frequently mutated, and they include

codon 175 (exon 5), codons 245, 248, and 249 (exon 7), and codons 273 and 282 (exon 8).

Mutations in the p53 gene are fairly common in dog cancer as well as human cancer. There have been numerous studies done on both human and canine p53, and also on how they are similar. Mayr et al. (1997) sequenced canine p53 exons to detect mutations of neoplasms that were in or near mutation hotspots. DNA was examined from 5 healthy tissue samples and 50 tumors, and two segments were amplified (Mayr et al., 1997). One segment corresponded to part of exon 5 and exon 6 (codons 132-200) and the other corresponded to exon 7 (codons 261-277). Out of 50 dogs with tumors, 49 of them had identical sequences, and only one showed a p53 mutation (Mayr et al., 1997). This mutation was a CGG→ TGG mutation in codon 249 of exon 7, from an adenoma of a circumanal gland. The researchers believe that the cancers that exhibited wild-type p53 would have a better prognosis for response to radiation therapy. Since radiation therapy damages DNA, any functional p53 might induce apoptosis and therefore kill the cancer (Mayr et al., 1997).

Leeuwen et al. (1997) examined canine osteosarcomas for p53 mutations in the region between exons 4 and 8. Almost 25% of all human osteosarcomas have a p53 mutation within this region (Leeuwen et al., 1997). Mutations in the p53 gene have also been identified in the germline of some humans with osteosarcomas who come from families with an inherited cancer predisposition (Leeuwen et al., 1997).

Spontaneous osteosarcomas in dogs share several features with spontaneous human osteosarcomas, including histology, biological behavior, and response to chemotherapy (Leeuwen et al., 1997). The most frequent site for canine osteosarcomas is

the appendicular skeleton, with tumors of the axial skeleton being less common. In this study, 18 dogs with osteosarcomas were examined for p53 mutations. If the tumors had metastasized, the metastatic tissues also were analyzed (Leeuwen et al., 1997).

Seventeen primary tumors and five metastatic tumors were studied, and of these four of the primary tumors exhibited mutations in one or both DNA fragments. Three of these mutations were missense, and two of them fell into the highly conserved region. One of the missense mutations was from a very young dog, indicating a possible germline mutation. Another mutation involved codon 273, which has also been found to mutate in human osteosarcomas and other human cancers (Leeuwen et al., 1997). It appears that the p53 gene mutates in both dog and human osteosarcomas at comparable rates, and also in the same region.

Johnson et al. (1998) examined exons 5-8 of the p53 gene in canine osteosarcomas. Fifteen dogs with appendicular osteosarcomas were looked at, and out of these, seven had point mutations (Johnson et al., 1998). One of these tumors had two mutations in two different exons. Seven of the mutations were missense mutations and one was a silent mutation. Five of the mutations were in the highly conserved region, while one was in codon 220, a mutation hotspot outside the conserved region (Johnson et al., 1998). These locations and types of mutations were nearly identical to data from previous human studies.

Most tumors with the p53 gene inactivated have both alleles inactivated, one usually by mutation and the other by deletion. Allelic deletion takes place in over 70% of human osteosarcomas. Other mutations, such as point mutations, small deletions, insertions, and splice mutations make up around 18% of the p53 mutations, while gross

rearrangement and partial deletions make up the remainder of p53 mutations (Johnson et al., 1998). The most common point mutation in human osteosarcomas is a G:C → A:T mutation. In humans, that makes up 47% of the p53 point mutations, and made up 62.5% of the mutations in this study (Johnson et al., 1998). Wild-type p53 was not present in most of the samples from this experiment, which provides strong evidence that mutated p53 genes play a role in osteosarcoma development similar to humans.

The experiment also found that reintroducing wild-type p53 into metastatic osteosarcoma cell lines in the lab led to apoptosis, causing the suppression of tumor growth (Johnson et al., 1998). It also caused increased tumor radiosensitivity, which could cause tumors to be more susceptible to radiation therapy. Humans and dogs share a number of similarities in osteosarcomas, including relative body mass, anatomic site, high metastatic rate, and prognosis, which could mean that they would also share a similar response to the reintroduction of wild-type p53. Canines would provide an excellent osteosarcoma study model. Cases would be readily available due to the fact that dogs are 40 times as likely as humans to develop an osteosarcoma, and their tumors also metastasize earlier (Johnson et al., 1998). In fact, canine osteosarcomas are one of the most biologically aggressive canine tumors, and often metastasize before a primary tumor is even identified.

Several studies have been done to sequence regions of the canine p53 gene. Kraegel et al. (1995) analyzed a region of the dog p53 gene corresponding to codons 25-312 in the human p53. The 828 base pair region was sequenced and found to be 83% homologous with the human p53. The conserved region is also fairly homologous with the cat and mouse p53 sequence. This study was important because it established the

normal sequence for canine p53, which is needed before abnormalities can be detected (Kraegel et al., 1995).

Mayr et al. (1999) looked at p53 in epithelial skin tumors and mammary neoplasms. Fifty dogs were used, and DNA was extracted from tumor specimens and peripheral blood immediately after the dogs underwent surgery (Mayr et al., 1999). The fifty neoplasms that were collected included 10 squamous cell carcinomas of the skin, 6 basalomas, 4 basal cell carcinomas, 4 sebaceous gland adenomas of the skin, 11 mammary adenomas, and 15 mammary adenocarcinomas. The researchers amplified four segments of the canine p53 gene between exons 4 and 8 and looked for mutations. Mutations were only found in three cases, which was an unexpectedly low rate. The first mutation was a one base pair deletion in codon 89 of a squamous carcinoma. The second mutation was in a mammary carcinoma and was a CGC→CAC point mutation in codon 162 of exon 5. This is of interest because canine codon 162 corresponds with the human mutation hotspot codon 175 (Mayr et al., 1999). The third dog had a TCC→TCT mutation in codon 103 of a mammary gland adenoma. This mutation appeared to be silent and have no role in tumor development. Based on the results of this study, the researchers did not feel that they had enough data to draw definitive conclusions about the role of p53 in canine skin and mammary tumors (Mayr et al., 1999).

The p53 gene has also been found to be involved in the predisposition for humans to develop breast cancer. Breast cancer is the most common cancer in women and, in most cases, is sporadic. There are two other important genes that can be involved in the development of breast cancer, BRCA1 and BRCA2 (Chu et al., 1998). It is possible that germline p53 mutations predispose humans to develop mutations in these two genes, or

vice versa, leading to an increased risk of developing breast cancer. Previous studies have shown that p53 mutations, as well as mutations in a gene called PTEN/MMAC, are found in many sporadic breast cancers, but little other progress has been made in determining a genetic factor. An appropriate animal model would be incredibly helpful in discovering more information about how p53 is involved in the development of breast cancer in humans (Chu et al., 1998).

Canine mammary tumors provide a good model for human study for several reasons. This is the most common cancer in female dogs, they share histological and biological features with human breast cancer, the relative age of onset is comparable between the two species, and tumor development in both species depends on sex steroid hormones (Chu et al., 1998). There is also some evidence that certain breeds develop breast cancer at a higher rate than others, pointing toward a genetic influence.

Chu et al. (1998) performed an experiment on canine breast cancer that indicated that p53 plays a similar role in dog and human breast cancer. Tumor specimens were taken from 52 dogs, including both primary tumors and distant metastases (Chu et al., 1998). The results of this study indicated that the exon and intron structure of the p53 gene was highly conserved between dogs and humans, and that the gene product was 81% identical. It was also found that there was a high level of homology in the four biggest mutation hotspots (Chu et al., 1998).

Six of the 40 primary canine tumors examined had a p53 mutation, and four carcinomas also had a p53 mutation in metastatic tissue. The benign tumors used did not show any p53 mutations (Chu et al., 1998). All of the mutations that were found were predicted to directly affect the p53 polypeptide coded for by the gene. This experiment

showed that at least 15% of canine mammary carcinomas have a p53 mutation, and the researchers felt that this was an underestimate. Relying on this rate, it appeared that the p53 gene mutated slightly less often in canines than it did in humans; however, additional data gathered from future studies would be beneficial in drawing more solid conclusions (Chu et al., 1998).

Some experiments on canine p53 have not provided any positive connection between tumor development in dogs and p53 mutations. For example, Mayr et al. (1999) examined four different tumors, including histiocytoma, granulocytic sarcoma, hemangiopericytoma, and mastocytoma. The researchers were looking for mutations in both the ras gene family and in p53 hotspots. Surprisingly, they found no mutations in either exons 1 and 2 of the ras oncogene or in exons 5-8 of the p53 gene. However, it is possible that the sample size for the study was too small or that there were mutations outside of the regions examined. It is also possible that p53 mutations do not play a role in the development of these four types of canine cancers.

Another possible explanation for the absence of p53 mutations in this experiment is that other genes could have been mutated that affected p53. For example, in humans, it is not uncommon for tumors to exhibit a normal p53 gene, but have a mutation in another gene that causes p53 transcription to be blocked or interferes with p53 protein activity (Mayr et al., 1999). These genes could cause a loss of p53 function and have the same result as a p53 gene mutation would. In this study, the levels of p53 RNA and proteins in the tumors were not known, so it is impossible to know if p53 was functional in these cancers (Mayr et al., 1999).

Another gene that is thought to affect the function of p53 is the mdm2 oncogene. Overexpression of mdm2 has the potential to inactivate p53, leading to uncontrolled cell growth and tumor formation (Nasir et al., 2001). The mdm2 gene is overexpressed in about 7% of human cancers, with the highest frequency of mutation found in soft tissue sarcomas, osteosarcomas, and esophageal carcinomas. The mdm2 gene is highly conserved between humans, mice, and canines, suggesting that it may play a significant role in canine cancer by inactivating p53 (Nasir et al., 2001).

Nasir et al. (2001) studied the effects of mdm2 mutations on p53 in dogs using samples from soft tissue sarcomas, which make up about 15% of all tumors in dogs. Forty tumor samples were collected, including 36 primary tumors and 4 metastases. Exons 5-8 of the p53 gene were successfully amplified in 34 samples, and none were found to have major deletions or insertions. However, gene mutations were found in 6 out of 30 primary tumors and in 1 metastasis, the majority of which were found in exon 8 (Nasir et al., 2001).

Amplification of the mdm2 gene was detected in 4 out of 35 primary tumors and in 1 metastasis. Additionally, 33 samples were examined for mutations in both the mdm2 and p53 gene. Out of these, 7 showed p53 mutations, and 5 of those also had amplifications in mdm2 (Nasir et al., 2001). This indicates that mdm2 amplifications are most likely responsible for affecting the p53 gene. It appears that mdm2 downregulates p53 by degrading or suppressing p53 transcriptional activity (Nasir et al., 2001). It has also been shown that tumors that contain both a p53 mutation and mdm2 amplification exhibit a worse prognosis than if they have either mutation alone. This experiment

proves that it is important to keep gene interactions in mind whenever studying the effects of p53 mutations in dogs.

In addition to other genes affecting the function of p53, it is possible for this gene to be affected by viruses. For example, one such virus that is present in humans is the papillomavirus, which is a species specific epitheliotropic virus that infects the skin and mucous membranes (Teifke et al., 1998). Humans that develop papillomavirus have a great chance of a malignant progression, because the papillomavirus degrades p53 and forces cells into replication cycles.

Dogs are also susceptible to papillomaviruses, with the most common type being oral viral papillomatosis (Teifke et al., 1998). Young dogs are especially prone to developing this condition, which consists of cauliflower-like masses on the tongue, lips, buccal mucosa, palate, pharynx, or gingiva. These masses are usually benign and regress spontaneously, but can develop into squamous cell carcinomas (Teifke et al., 1998).

Teifke et al. (1998) studied canine p53 and papillomavirus by examining 19 papillomas, 29 oral squamous cell carcinomas, and 25 non-oral squamous cell carcinomas. Abnormal p53 activity was found in about 35% of the squamous cell carcinomas, and papillomavirus was found in approximately 50% of the oral and non-oral papillomas. Papillomavirus was also found in three oral squamous cell carcinomas, which indicates that progression from papillomas to squamous cell carcinomas does occur in dogs (Teifke et al., 1998). However, it is not clear whether or not the abnormal p53 is dependent on a papillomavirus being present like it is in humans. It is possible that p53 is inactivated by a multistep progression from a papilloma to a squamous cell

carcinoma (Teifke et al., 1998). Further studies are needed to determine whether or not canine papillomavirus has the capability to inactivate p53.

Metastasis-Affecting Genes

V-src

There appears to be a third category of genes that affect canine and human cancer that are neither proto-oncogenes or tumor-suppressor genes. These genes influence whether or not certain types of cancers metastasize. One of these genes is src, called v-src when mutated. The src gene normally plays an important role in cell differentiation, proliferation, and survival. It has also been found to be involved in cell adhesion, cell morphology and motility, and bone resorption (Roskoski, 2004). Noritake et al. (1999) studied the effects of mutated v-src by examining canine kidney cells and especially focused on the possibility of metastasis in these cells. The process of a tumor metastasizing requires the malignant tumor cells to invade the surrounding cells to form colonies (Noritake et al., 1999). In order for this to happen, the extracellular matrix and basement membrane must be degraded in the cancerous cells. It is necessary for proteolytic enzymes to be present to degrade these materials. In many human tumors, proteolytic enzymes called matrix metalloproteinases are overexpressed (Noritake et al., 1999).

Matrix metalloproteinases are secreted as inactive zymogen, and activation is the key step necessary to begin the degradation and thus the metastatic process (Noritake et al., 1999). Tumors are very conducive sites for the activation of these matrix metalloproteinases. Noritake et al. (1999) forced canine kidney epithelial cells to express

MT1-MMP, a matrix metalloproteinase, by transforming them with a mutated v-src gene. The kidney cells, which were tumorigenic, spontaneously metastasized to the lungs.

Interestingly, another gene found to be overexpressed in the v-src mutated kidney cells was TIMP-1 (Noritake et al., 1999). This gene in humans is generally thought to inhibit matrix metalloproteinases, but does not work on MT1-MMP. The canine TIMP-1 gene is 81% homologous with the human TIMP-1 gene, and therefore it could be used as a good research model (Noritake et al., 1999). It is possible that the reason these cancerous cells are able to metastasize is because they are producing an elevated amount of MT1-MMP, which cannot be regulated by TIMP-1. Normal kidney cells showed no proteolytic activity, but the cells with mutated v-src digested the extracellular matrix fairly easily. It can therefore be determined that MT1-MMP plays a significant role in the degradation of the extracellular matrix (Noritake et al., 1999). Future research on the similarities between canine and human v-src genes may be beneficial in providing knowledge about the mechanism of metastasis.

Mts1

Another mechanism by which cancer cells can become metastatic is via the overexpression of the mts1 gene. The normal function of the mts1 gene is to encode a calcium binding protein, metastasin, which is capable of regulating the invasive and metastatic properties of cancer cells (Miyamori et al., 2000). Studies have shown that MT1-MMP can turn stationary cells into highly metastatic cells, but the overexpression of mts1 also apparently has a significant impact. Miyamori et al. (2000) demonstrated that MT1-MMP and the mts1 gene were co-expressed during the oncogenic transformation of canine kidney cells. It appears that the two genes may cooperate to

create an invasive phenotype for cancerous cells. This study shows that the level of mts1 RNA is correlated to the level of MT1-MMP, and the two are likely co-induced. The canine mts1 gene has a high level of homology (roughly 95%) with the human mts1 gene (Miyamori et al., 2000). It may be possible in the future to determine a way of controlling mts1 expression in dogs, and therefore apply that to human mts1, helping control metastasis of cancers in both species.

Important Genes

As many studies have shown, there are several canine genes that can provide significant information for the study of both human and canine cancers. These genes are:

Proto-oncogenes

k-ras	HER-2
c-kit	HMGA1
c-yes	

Tumor-Suppressor Genes

p53

Metastasis-Affecting Genes

v-src
mts 1

Conclusions

There are likely many more genes that are highly homologous between canines and humans that have not yet been discovered. Currently, there is fairly limited

information available on the canine genome itself, and even less on its mutations. I believe that the dog will become an increasingly important model for cancer research as more cancer-related genes are discovered, and will be especially useful because its genes are so similar to a human. Even in cancer-related genes that are not extremely homologous between the two species, it seems that research done on canines would provide a good starting point for human research.

Dogs provide an excellent study model not only because of their genetics, but also because they live in an environment almost identical to most humans, which is a relatively unique feature among animals. In addition, many advances in canine genetics have been made recently, so it is not unrealistic to envision major advances in cancer research in the near future. Already, retroviral and adenoviral vectors have been used to successfully transfer gene constructs to several canine cells, including fibroblasts, hepatocytes, and thyroid follicular cells (Kruth, 2004). Also, canine hemophilia B and mucopolysaccharidosis I are currently being used as models for human gene therapy. Many oncologists feel that several human cancers have the potential to benefit from canine gene therapy models, including lymphomas, osteosarcomas, and melanomas (Kruth, 2004). As data on canine genes become more available due to future research, I believe dogs will emerge as significant models for human cancer treatment studies.

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