

Kingfisher Biotech Circular

Subject: Canine as an Animal Model

Volume 1; No. 1

Domesticated dogs are increasingly being identified as good models for a variety of biomedical research fields as they have a number of unique advantages over other commonly used experimental animals. Perhaps the most interesting of these is the fact that dogs cohabitate with their owners, are kept into their old age, and generally receive a good level care including highly-trained healthcare. This modeling of the human condition offers valuable opportunities for researchers to examine complex problems such as environmental contributions to diseases, aging and its effect on disease susceptibility and progression, and the effects of long-term treatment protocols. [1-3]

Another advantage is that over the past several centuries, domestication and selective breeding of dogs has resulted in nearly 400 distinct populations and thus the most naturally occurring genetic diversity in any one species besides humans. Careful breeding for trait selection has inadvertently resulted in breed-specific disease susceptibilities and approximately 400 naturally-occurring, inherited diseases have been identified in dogs. Most predicted canine genes have known human homologs and several of these heritable canine diseases have been associated with mutations in canine and human homologous genes. The enormous genetic diversity of canine breeds (many of which have extensive pedigree information) and the broad range of spontaneously-occurring canine diseases afford researchers opportunities to examine genetic etiologies and explore the possibility of gene therapies. Unfortunately, this genetic diversity is also the main disadvantage of canine models as there are breed-specific differences in physiology and metabolism, especially idiosyncrasies in pharmacodynamics and pharmacokinetics, which can introduce complications when interpreting or translating results. However, increasing research in this area is expanding on our current understanding of these issues. [1-3]

Finally, due to the rapid aging of dogs, there is a shorter duration for disease development and progression. This is an enormous advantage in the context of drug development as clinical trial study times are significantly reduced. Potential for rapid study times along with reduced regulatory guidelines and the increasing acceptance of canine models of human diseases by regulatory bodies is resulting in the growing use of dogs as models for translational medicine. Canine models have already served to advance human medicine in a number of areas and have been instrumental in some, such as narcolepsy, hemophilia, retinal degeneration, and muscular dystrophy. [1-3] A brief overview of a selection of biomedical research fields in which canine models are used is described here. This summary should be taken as neither comprehensive nor definitive.

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Cancer

Dogs are particularly good models for the study of cancer. They spontaneously and with high frequency develop the same types of cancers that humans do and are often even treated with the same therapeutic strategies. Additionally, the centuries of selective breeding of dogs confers opportunities to examine polymorphisms specific to particular breeds that have exaggerated incidences of cancer subtypes. Finally, because dogs cohabitate with their owners, they are both exposed to the same environmental factors that may potentiate the development of cancer. This offers an exceptional opportunity to look at the interactions between genetics and environment in the etiologies of various forms of cancer. A wide variety of cancers are being studied in dogs including soft tissue sarcomas, mammary carcinomas, primary and secondary lung carcinomas, malignant melanomas, and cancers of the prostate, bladder, intestine, brain, mouth, and many others. In the interest of brevity, only two forms of cancer for which canine models are used will be highlighted here. [1,4]

Non-Hodgkin's Lymphoma (NHL) - Approximately 5% of all human cancers are one of several forms of lymphoma. Non-Hodgkin's lymphoma (NHL), in particular, is increasing in incidence yet is still of unknown etiology. Dogs are an excellent model for human lymphomas as they spontaneously develop the disease with high frequency, accounting for nearly 25% of all life-threatening canine cancers. In dogs and humans, NHL is most commonly the diffuse large B-cell variety and is treated with the same therapeutic strategies. The canine breeds with disproportionately high incidences of diffuse large B-cell type NHL are Boxers and Golden Retrievers. Research using these breeds has led to a better understanding of lymphoma cytogenetics, which suggests the pathogenic origin of lymphoma is very similar between humans and dogs. NHL-afflicted dogs serve as models for the testing of new drugs, drug delivery methods, and immunotherapies, as well as modified diets and other treatment strategies. For example, preclinical testing of a selective, irreversible inhibitor of B-cell activation and an anti-human leukocyte antigen (HLA) monoclonal antibody are ongoing and showing promising results so far. [1,4]

Osteosarcoma (OSA) - Although relatively rare in humans, osteosarcoma (OSA) is of particular interest as it mainly affects children and adolescents and is very aggressive (80% metastatic rate) and notoriously resistant to chemotherapy (more than 30% of patients are unresponsive to available drug regimens). Sadly, the 5-year, post-diagnosis survival rate for metastatic OSA patients is only 20%. Like human OSA, canine OSA is most common in heavy individuals, is generally confined to bone (particularly long bones), and has similar metastatic rates (90% in dogs) and destinations (lungs and soft tissues). Large breeds such as Great Dane, Wolfhound, and Rottweiler are most susceptible to OSA and genetic features found in OSA-afflicted individuals of these breeds appear to be reflected in human OSA sufferers as well. For example, both human and canine OSA sufferers display similar alterations in the p53 tumor suppressor pathway. Candidate genes identified in both humans and dogs include the c-Met proto-oncogene (*a.k.a.* hepatocyte growth factor [HGF] receptor), the chemokine

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IL-8 (*a.k.a.* CXCL8), and several others. Elevated, co-expression of both HGF and c-Met is observed in both human and canine OSA tumors and in humans is associated with increased tumor growth, invasion, and metastasis. Over-expression of IL-8, is observed in canine OSA tumors and in human tumors from patients with especially poor outcomes. Closer examination of these and other candidate genes in dogs and in humans will undoubtedly lead to a better understanding of OSA pathophysiology and more effective, targeted therapies. [1,2,4]

Aging & Alzheimer's Disease

There are significant breed-specific differences in canine longevity – smaller breeds having longer lifespans and larger breeds having shorter lifespans. Generally, the Beagle has been selected as the main breed used for aging studies as its median lifespan is 12 to 14 years, and Beagles over 9 years of age are considered “old,” representing humans aged 66 to 96 years. Both cognitive and neuropathological changes in aged Beagles have been well documented and seem to closely approximate human clinical observations in many aspects. These features make dogs, and in particular Beagles, well-matched for studies of human aging and age-related conditions such as Alzheimer's disease (AD). [5]

Like humans, aged dogs naturally suffer from AD that is characterized by the deposition of significant amounts of Amyloid β ($A\beta$) protein and the development of diffuse plaques, the extent of which quantifiably correlate with cognitive decline. However, AD-afflicted dogs do not appear to naturally develop neurofibrillary tangles as observed in humans. This may be explained by the fact that while the $A\beta$ amino acid sequence is identical in dogs and humans, the Tau amino acid sequence is appreciably different in the two species. It has been suggested that presence of $A\beta$ deposits and plaques and lack of neurofibrillary tangles represents early stages of AD, which may uniquely position the canine AD model for investigations into the possibilities of preventative measures and early interventions. Therapeutic strategies under investigation using canine AD models include antioxidant diets and behavioral enrichment. Both of these regimens have been shown to improve AD pathology when delivered individually and even more so when administered in combination. Other treatment strategies being pursued in dogs include disruption of $A\beta$ processing by anti-inflammatory or statin drugs and immunization against $A\beta$ peptide. [5,6]

Respiratory Disease

Dogs have been commonly used for the study of human respiratory system function and dysfunction and as a consequence, an extensive body of literature describing canine pulmonary anatomy and physiology already exists and includes data related to lung mechanics, ventilation, and cough reflex, immunobiology and inflammation, pharmacology, and central neuronal control mechanisms. Further, their large size and easy handling make dogs ideal for long-term studies of the effects of inhaled irritants such as common components of air pollution (*i.e.*, ozone or SO_2) and cigarette smoke.

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There are many similarities between human and canine respiratory systems and dogs have been used particularly as models for chronic inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD), which develops from chronic bronchitis and emphysema. [7]

Asthma - Asthma induced in healthy canine subjects via challenge with antigen, hyperventilation, or ozone results in classical characteristics of asthma in humans - airway hyperresponsiveness and chronic inflammation that results in bouts of wheezing, chest tightness, breathlessness, and coughing. While canine pulmonary responses to each of these asthma-inducing methods are slightly different in some aspects, the commonalities are significant and closely parallel clinical observations of patients with asthma. In general, dogs with induced asthma acutely display changes in the mechanics of airway constriction and increases mast cell mediators including histamine, prostaglandin D₂, thromboxanes, serotonin, and vasoactive peptides. This is associated with significant damage to airway mucosa and some of these mediators (*i.e.*, histamine, serotonin, and bradykinin) are known to directly stimulate sensory afferents in the lungs and subsequently affect central control of airway function. Chronically, dogs with induced asthma display airway hyperresponsiveness and chronic inflammation. Airway hyperresponsiveness is characterized by rapid bronchoconstriction at magnitudes 2 to 10-fold greater than normal and by destruction of airway epithelium possibly as a result of reduced bronchoprotective prostaglandin E₂. Asthma-associated, chronic inflammation in lung tissues is characterized by neutrophil, eosinophil, and T lymphocyte infiltration as well as mobilization of new bone marrow progenitor cells to lung tissues. These canine models of induced asthma display many similarities with human asthma and serve as good models for the study of the disease and potential therapeutic strategies. [7]

Chronic Obstructive Pulmonary Disease (COPD) - Although dogs naturally develop both chronic bronchitis and emphysema, COPD is most typically studied in induced models - where normal healthy dogs are subjected to inhalation of cigarette smoke, SO₂ gas, or aerosolized proteolytic enzymes. In general, short-term exposure to these inhaled irritants results in pulmonary reflexes such as increased cough frequency and mucous production as well as bronchoconstriction. Further, there is evidence of edema, inflammation characterized by neutrophil and macrophage infiltration, alteration of airway mucous glands, and damage to airway epithelium. These pathological changes in the lungs closely resemble human chronic bronchitis. Longer-term exposure, in some cases, results in pathological changes in lung tissues resembling emphysema - alveoli enlargement and destruction and remodeling of lung parenchyma. [7]

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Endocrinopathies

A variety of endocrine disorders known to affect humans also occur spontaneously in dogs. Some examples of such endocrinopathies include diabetes, growth hormone (GH) dysfunctions (*i.e.*, dwarfism or acromegaly), and hypercortisolism (*a.k.a* Cushing's disease). Canine diabetes is caused either by autoimmune destruction of pancreatic β -cells or excess in counter-regulatory hormones (*i.e.*, hypercortisolism). Pituitary dwarfism in dogs is caused by autosomal recessive combined pituitary hormone deficiency (CPHD), which is often observed particularly in the German Shepherd breed. Canine CPHD is characterized by combined reduced levels of GH, thyrotropin, prolactin, and gonadotropins due to poor development of the pituitary gland and may be a good model for human CPHD. GH excess in dogs often leads to canine acromegaly. In this context, progesterone induces excess GH production in the mammary gland. This mechanism is also associated with up-regulation of insulin-like growth factor (IGF) and IGF binding protein (IGFBP) production. Together, these alterations in normal physiology in canine mammary tissue promote proliferation and appear to play a role in the development and/or progression of canine mammary tumors and hence may offer some insight into human breast cancer. [8]

Cushing's Disease - In humans and dogs, Cushing's disease is caused by adrenal cortex over-production of glucocorticoids (GCs). Endogenous excess GC production is most often the result of pituitary adenoma (80 to 85% of cases) and less often the result of adrenocortical adenoma (15 to 20% of cases) in both species. Although relatively rare in humans, middle-aged to old dogs commonly develop Cushing's disease with manifestations very similar to clinical observations in humans including changes in the skin, weight gain and abdominal obesity, fatigue and muscle atrophy, and hypertension and renal dysfunction. Because of these similarities between canine and human Cushing's disease, the high frequency of spontaneous disease in dogs, and the fact that canine pituitary and adrenal glands are relatively large (*i.e.*, compared to smaller animal models) and therefore easier to handle for histological, *in vitro*, and *ex vivo* studies make dogs excellent models for this disease. [8,9]

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