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CANINE COMPARATIVE ONCOLOGY IN THE POST GENOMIC ERA

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Release of the Canine Genome Sequence.

The public release of a high-quality sequence covering 99% of the canine genome (2.5 billion base pairs) in 2005 was received with great anticipation by several communities interested in the health and well being of pet dogs and/or potentially the expanded role of the dog in biomedical and translational research. The strong anatomic and physiologic similarity between dogs and humans has and continues to be the basis for the inclusion of the dog in many facets of biomedical research. The companionship provided by pet dogs is evident by the 72 million pet dogs in the United States, with similar per capita populations in much of the Western world, and the size of the pet dog health market (estimated to be \$42 billion/year in the United States). The completion of the canine genome has brought a greatly needed reagent to these communities. Most interesting from the canine genome are the remarkable similarities between the genomes of the dog and man. These include evidence that sets of functionally related genes show high evolutionary similarity in the dog and human. Furthermore, the current canine single nucleotide polymorphism (SNP) map is of adequate size and polymorphism rate to allow for gene-association studies to be conducted in the dog. ***Thus the post-genomic status of the dog will likely accelerate our knowledge of health and disease in dogs.*** Tied to this opportunity for accelerated discovery are many technological advances that provide the same high-throughput methodologies in dogs that have been used to understand human disease conditions. The creation of a commercially available Canine Genome oligonucleotide array, first by Affymetrix and now by others, as well as the more recently release canine SNP arrays, have made possible for high throughput interrogation of canine health and disease conditions. Work by several groups has begun to use the canine genome as a means to understand basic biological principles and genetic conditions.

Opportunities for Cross-Species Studies in Comparative Oncology.

The field of comparative oncology is uniquely positioned to take advantage of the completed canine genome to improve our understanding of cancer therapy and biology. Used most often when referring to the study of cancers seen in companion (pet) animals, comparative oncology describes a discipline that integrates the study of naturally occurring cancers in animals into studies of human cancer. Cancers in companion species are well suited to uniquely inform investigations of cancer biology and cancer drug development. The features of cancers in pet dogs that may uniquely contribute to our understanding of cancer pathogenesis, progression and therapy have been recently reviewed. Pet dogs are large and are relatively out bred in comparison to laboratory animals. Cancers developing in these animals are naturally occurring and develop in the context of an intact immune system where tumor, and host and tumor microenvironment are syngeneic. Tumor initiation and progression are influenced by similar factors in both human and canine cancers, including age, nutrition, sex, reproductive status, and environmental exposures. The biological complexity of cancers in pet animals captures the essence of cancer in human patients. This is based in large part on the intra-tumoral (cell-to-cell) heterogeneity seen in these cancers. A natural consequence of this heterogeneity is the same deadly features of human cancers including acquired resistance to therapy, recurrence, and metastasis.

Application of the Cross-Species Approach to the Problem of Cancer Metastasis.

Our laboratory has utilized a cross-species approach in the area of comparative oncology to improve our understanding of the biology and therapy of osteosarcoma. The development of metastasis is a universally grave development for cancer patients irrespective of specific cancer histology. For both human and canine cancer patients afflicted with osteosarcoma, the most common primary tumor of bone, metastasis to the lungs is the most common cause of death. Lung metastases develop in these

patients despite highly effective treatment of the primary tumor. Improving our understanding of the biology of metastasis as a process (verb) and as a clinical entity (noun) is needed to improve outcomes for these patients. Progress towards understanding metastatic disease and its inherent resistance to conventional treatments is limited by many factors. First, the process of metastasis is believed to begin very early in the course of disease progression and has occurred in most patients at the time of their initial presentation. This provides limited opportunities to study these events in human patients. Second, the genetic aberrations that are responsible for the development of metastasis are complex, heterogeneous and difficult to distinguish from the events responsible for the actual development of cancer. Finally, the access to well described patient samples is limited and often is available only after treatment with cytotoxic chemotherapy. For relatively rare cancers such as osteosarcoma, these problems are amplified.

To define genes and or proteins that contribute to the metastatic phenotype of metastasis in osteosarcoma, we have utilized a cross-species comparative approach that includes murine, canine, and human systems for gene identification and evaluation. This approach has been based on the use of novel non-candidate studies, leveraging the availability of murine, canine, and human genomes, to survey the expression of genes in normal and diseased tissues and then identify either patterns of gene expression of individual genes responsible for or associated with metastasis. As an example, we used this comparative approach to identify and then associate the cytoskeleton linker protein, ezrin, with metastasis. The stability and consistency of our findings involving ezrin across species lines has strengthened our belief in this cross-species approach. We first identified ezrin using cDNA microarrays and a metastasis based methodology for array evaluation. Ezrin's linkage of the cell membrane to the actin cytoskeleton directly allows the cell to interact with its micro-environment and functionally provides an "intracellular scaffolding" that facilitates signal transduction through a number of growth factor receptors and adhesion molecules. Ezrin is the best characterized of the ERM (Ezrin-Radixin-Moesin) family. ERM proteins exist in the cytoplasm in an inactive "closed conformation" though N-terminal to C-terminal associations within the protein or with other ERM members. We have demonstrated that ezrin is necessary for metastasis in murine transplantable osteosarcoma and genetically engineered rhabdomyosarcoma models, that it is relevant in human-murine sarcoma xenograft models, and that its expression is associated with metastatic progression in pet dogs with naturally occurring osteosarcoma, finally we have found an association between ezrin expression and risk of relapse in pediatric osteosarcoma patients. The value of this comparative approach has been to define biologically relevant motifs that have sustained importance as we cross species lines.

Evaluating Antimetastatic Therapies Based on Ezrin Biology.

The comparative approach is uniquely suited to rapidly translate findings from the laboratory to the clinic. With this interest in mind we have recently linked ezrin expression and the mTOR (mammalian target of rapamycin) pathway in the translation of proteins by metastatic cells. We have begun to validate these associations in murine, canine and human cancer cells and have conducted preclinical studies in mice that support the therapeutic role of inhibiting mTOR using rapamycin (and novel analogs). Indeed novel inhibitors of the mTOR pathway have entered early clinical trials for human osteosarcoma and sarcoma patients. There are many questions that remain unanswered regarding the optimal use of these agents in human patients. These include the optimal dose, schedule and regimen for therapy, the appropriate combinations of existing and novel cancer treatments with mTOR pathway inhibitors, and the development of biomarkers of exposure and response to therapy. Clinical trials in client owned dogs are not constrained by traditional Phase I, Phase II and Phase III trial designs. Novel agents can be offered to pet dogs as single agents before any conventional treatment has been provided or during the period of minimal residual disease. Moreover, such agents can readily be added to conventional treatment regimens such as chemotherapy and radiation therapy to determine optimal therapeutic combinations. Accordingly many of the unanswered questions that exist in the development path of new drugs may be effectively answered by integrating studies that

include pet dogs with cancer. To complete the translational effort based on ezrin and mTOR inhibition, we have now completed dose and regimen finding studies in pet dogs with osteosarcoma that will allow the evaluation of the inhibition of translation initiation (by rapamycin) in pet dogs with osteosarcoma. Ongoing studies will now define optimal treatment schedules for the use of rapamycin in children and dogs with osteosarcoma. Efforts to validate reagents and further characterize these models using more sophisticated techniques has been ongoing within several comparative oncology laboratories around the world. Contributing to this effort is Comparative Oncology Program of the US National Cancer Institute's Center for Cancer Research and the not for profit, Canine Comparative Oncology and Genomics Consortium.

Extending the Opportunity of the Cross Species Approach.

Based on the genomic opportunities provided by the completion of the canine genome, the identical biological behavior of canine and human osteosarcoma, and the increased prevalence and aggressiveness of this disease in dogs we recently used our cross-species comparative gene expression approach to uncover specific genes, gene families/functions, or pathways that were conserved across the dog and human and are commonly linked to metastasis. Using identical oligonucleotide microarray platforms we compared expression signatures for primary tumors and normal tissues from both dogs and humans. Rather than finding clear similarities and differences between the canine and human cancers, the similarities between canine and human osteosarcoma were so strong, that cluster analysis of 265 orthologous transcripts could not distinguish the canine and human cancers. Based on the surprising resemblance between the expression profiles of osteosarcoma in human and dog we then asked whether the aggressive biology of the dog disease could help identify genes important in metastatic progression of osteosarcoma that would have been overlooked if the human disease was studied alone. Four genes were identified from 15, that were consistently overexpressed in canine osteosarcoma ("dog-like" genes) but variably expressed in humans. Using a naive human expression data set of human osteosarcoma, that was linked to outcome, we found that two of the "dog-like" genes were associated with a more aggressive clinical course in patients. Statistical comparison to random selection approaches suggested this to be unlikely the result of chance alone.

Collectively, our comparative approach has provided a novel, necessary and informative perspective for the study of cancer biology. The approach exemplifies the values of One Medicine in the study of complex biomedical problems.

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