Update on AKC CHF Grant 01113:

Canine Non-Hodgkin Lymphoma: Characterization and Prognostic Value of Cancer Stem Cells

Principal Investigator: Dr. Timothy D. O'Brien, DVM PhD, University of Minnesota

Report to Grant Sponsor from Investigator:

Enumerate cancer stem cells and endothelial precursor cells in archived and freshly isolated samples of canine non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) represents a heterogeneous group of tumors divided into more than 25 subtypes based on cell lineage and histological morphology. Histological classification and clinical stage are used to predict patient outcomes; however, disease progression is variable even within the same NHL subtype, and therefore additional means of establishing prognosis and outcomes are needed. The growth of small blood vessels into tumors (angiogenesis) has been correlated with the extent of disease and progression of lymphoid tumors in dogs and humans, but the prognostic value of mature endothelial cells (cells which form the lining of blood vessels/ECs) and endothelial progenitor cells (immature cells which may proliferate to form additional endothelial cells/EPCs) as surrogate markers for angiogenesis in canine NHL is unclear. Furthermore, the significance of lymphoid progenitor cells (immature cells which may proliferate to form lymphocytes/LPCs) in this disease has not been addressed. Here, we evaluated the predictive value of ECs, EPCs and LPCs in NHL dogs.

We identified cells that co-expressed protein markers that identify EPCs; LPCs or ECs, in blood and lymph nodes from dogs with NHL treated using standard of care multi-agent chemotherapy. The normalized frequency of ECs, EPCs and LPCs was compared with patient outcomes.

Our investigations showed that LPCs occur in all canine lymphomas at levels far above those in normal lymph nodes and additionally that the LPCs carry markers of lymphoid differentiation and also carry the same gene rearrangement as the bulk of the tumor cells. These findings indicate that the LPCs are indeed likely the progenitor cells of the tumor. Our results furthermore suggest the presence of a hierarchy of tumor cells in canine NHL as has been demonstrated in other cancers. By establishing the identity of the LPCs we have accomplished the critical first step in understanding this basic aspect of lymphoma. Further understanding of the biology of LPCs may inform the development of more effective treatments for canine NHL.

Define the phenotype of putative cancer stem cells in non-Hodgkin lymphoma

Stem cells, such as the well-characterized embryonic stem cells, have been shown to have characteristic patterns of gene expression. Similar patterns of "stem cell marker" genes have also been shown to occur in cancer stem cells. Therefore, one approach to evaluating the putative cancer stem cells that we have identified in dog lymphomas is to look for expression of specific genes that are known to occur in various types of normal stem cells. Toward this goal we analyzed expression of stem cell-related transcription factors which are known as pluripotency and self renewal factors in normal or inducible stem cells. The research results showed the LPC population of cells had markedly greater expression of a hematopoietic stem cell marker protein versus the bulk tumor cells consistent with the LPCs being a cancer stem cell. The findings also show that the LPCs do not express high levels of other proteins as seen in populations of pluripotent stem cells such as embryonic stem cells, but are more similar to adult stem cells such as hematopoietic or neural stem cells in this regard.

The second method by which we proposed to evaluate the phenotype of the lymphoma stem cells was DNA microarray analysis which allows the simultaneous assessment of expression of tens of thousands of genes. This assay is less sensitive but is a powerful way to assess overall features of gene expression in cell populations. Toward achieving this goal we prepared RNA samples from LPCs and bulk tumors from several dogs. Now the vast amount of data generated from the microarrays will require extensive analysis which will be ongoing for the next several months.