Abstract of Proposed Student Project

Title: Early Detection of Cancer in Companion Dogs Using Liquid Biopsy

Background: Circulating Tumor DNA (ctDNA) is a cell-free DNA that is released from tumor cells and shed to the systemic blood circulation. ctDNAs can be identified in the plasma and sera acquired from cancer patients, and they may contain tumor-specific mutations that at least partly manifest genomic features of primary or metastatic tumors. Human cancers appear to spread substantial amounts of fragmented genomic DNA that are associated with tumor size and clinical outcome, suggesting that it has potential diagnostic and prognostic implications. Cell-free DNAs are also detectable in canine tumors, and it is suggested that the quantification of ctDNA provides potential clinical values. Yet, cell-free DNAs have been evaluated in only a limited number of canine samples, and clinical implications of cell-free ctDNAs remain unclear in canine tumors.

In this project, we hypothesize that circulating cell-free DNAs identified in the peripheral blood will capture genetic and molecular features of canine tumors informing disease progression and clinical outcomes. Specific aims of this project are **1) to isolate and characterize cell-free DNAs in the peripheral blood of dogs with hemangiosarcoma, osteosarcoma, and mast cell tumors** with or without sentinel lymph node involvement, and **2) to develop a machine learning-assisted approach to determine pathological significance of cell-free DNA profiling data in the ontogenetically distinct tumors**.

Experimental approach: We will collect blood from dogs with hemangiosarcoma (N=20) and osteosarcoma (N=20) without metastatic disease at the time of diagnosis, and dogs will be monitored to assess clinical outcomes after surgical tumor removal. Blood samples from canine mast cell tumors with sentinel lymph node involvement (N=20) and without that (N=20) will be also acquired. Blood obtained from healthy dogs (N=20) will be used as controls. We will isolate cell-free genomic DNA in sera and plasma samples obtained from dogs with those tumors. Cell-free DNAs will be quantified and characterized by PCR and qPCR. Then, we will generate next-generation sequencing to identify mutations in cell-free DNAs, along with PCR validation as well as to establish high-throughput genomic and molecular profiles. For machine learning applications, 10-fold cross validation will be done to test a prediction performance of multiple machine learning algorithms (at least 10 different classifiers). By completing training and validation process with feature selection, tumor-associated circulating molecular signatures will be identified as potential biomarkers for tumor detection and disease progression.

The FSVP student's role: The student will learn how to isolate and purify cell-free DNA from canine sera and plasma samples. Basic molecular techniques such as PCR and qPCR with Sanger sequencing validation are necessary to optimize protocol for isolation of cell-free DNA and to perform quality control process. The student will help prepare samples and reagents and learn how to design PCR primers and detect nucleic acids. The student will acquire basic knowledge of cancer biology including oncogenic mechanisms of hotspot tumor driver mutations such as *TP53* and *PIK3CA* gene. The FVSP student will work closely with our research team and will be trained by post-docs in our lab, Drs. Don Lee and Md Abdullah during the summer research period.