NEW INVESTIGATOR AWARDS

Richard Dahl, Ph.D.
University of New Mexico
$75,000.00 - ***Ets and Gfi1 family interactions in T cell development***

T and B cells comprise the adaptive arm of the vertebrate immune system. These cells arise from the hematopoietic stem cell and become committed to their respective cell fates through a series of differentiation steps that involve the progressive narrowing of their developmental potential. It has been shown that combinations of lineage specific transcription factors program hematopoietic stem and progenitor cells to become the mature cells of the blood system. This developmental program is disrupted in leukemias and lymphomas and hematopoietic transcription factors are frequently targeted to become oncogenes through translocations and somatic mutations. In this project we focus on the interactions between members of two transcription factor families, Gfi-1 and Ets. Gfi-1 members have been shown to be involved in the development of T lymphoma and Ets family members have been implicated in several leukemias and lymphomas. We have recently observed that Gfi-1 can interact with Ets family member PU.1 and repress PU.1's ability to activate transcription and induce differentiation. The hypothesis of this proposal is that Gfi-1s regulate Ets factor activity through protein-protein interactions and this regulation is critical for proper T cell development and function. We will first determine whether Gfi-1 antagonism of PU.1 and the related Ets factor Spi-B is important for proper T cell development. PU.1 and Spi-B are expressed in early T cell development and must be turned off in order for T cell maturation to proceed. We will investigate whether Gfi-1 is important in shutting off the activities of PU.1 and/or Spi-B. Secondly we will determine what other T cell expressed Ets family members can interact with Gfi-1 proteins. The ability of Gfi-1 proteins to bind to T cell expressed Ets proteins will be determined. Additionally we will investigate whether Gfi-1 proteins can affect the expression of T cell specific genes which are known to be regulated by Et family members. The results of this project will determine whether regulation of Ets family members is critical role for Gfi-1 proteins in T cell development and demonstrate whether misregulation of Ets family members could be a defect in Gfi-1 induced lymphomas.

Wladyslaw Krajewski, Ph.D.
Institute of Developmental Biology, Russian Federation
$49,500.00 - ***Interaction of MLL and related SET-domain proteins with active chromatin structures***

A typical human cell contains more than 30,000 genes, however only a few percent of them work in each particular cell. It is essential not only to keep the right genes on but also to keep the right genes off. The Polycomb and trithorax groups of proteins are the cell components that ensure correct states of gene activity. In general, Polycomb proteins stably repress genes in cells where these genes must remain inactive, while trithorax proteins maintain active gene states. Mistakes of this system have severe consequences. For example, misregulation by MLL, the human trithorax group protein, results in aggressive lymphoid and myeloid acute leukemias in both children and adults. The molecular principles of these events are essential not only for therapy of leukemias, but also for understanding the basics of tumorigenesis in general. However, this knowledge is limited. The Polycomb and trithorax group proteins are present in all cells but they selectively repress or activate each particular gene only in cells descended from cells in which such repression/activation was initiated in the early development. We propose a set of experiments designed to approach molecular mechanisms of how the Polycomb and trithorax proteins can recognize such predetermined gene activity states, to access if this proteins can selectively recognize and bind specific DNA structures, typical for active genes, whether the occurring *in vivo* unusually structured DNAs can participate in such regulation.

Alberto Martin, Ph.D.
University of Toronto
$75,000.00 - *The role of AID targeting in antibody diversification and lymphomagenesis.*
The immune system plays a major role in protecting humans from bacteria, viruses, and toxins. One of the ways that the immune system accomplishes this is by producing antibodies that bind to and neutralize these pathogens. B cells produce pathogen-specific antibodies and can increase the affinity of the antibody to the specific antigen by mutating the antibody genes. This process is termed somatic hypermutation and is initiated by an enzyme called activation-induced cytidine deaminase (AID). AID mutates antibody genes, but sometimes also makes mistakes and mutates other genes. When these other genes are mutated, these B cells can become lymphomas. Three types of lymphomas might be caused by mistakes made by the somatic hypermutation process, namely diffuse large cell lymphoma (DLCL), chronic lymphocytic leukemia (CLL), and Burkitt's lymphomas (BL). The overall goal of this research proposal is to determine what factors restrict AID to mutate antibody genes and why does it make mistakes and mutate other genes. With this work, we hope to understand the mechanisms of lymphoma development with the hopes of eventually developing therapeutic and/or diagnostic strategies.

Qishen Pang, Ph.D.
Cincinnati Children's Hospital Medical Center
$75,000.00 - *Inhibition of p53 by NPM and its relevance to leukemic development*
Most human cancers, including blood cancers such as leukemia, share two abnormalities: uncontrolled cell growth and a prolonged cell lifespan. One critical factor guarding our body to prevent these two cancer-developing events is the protein called p53. Although most solid cancers demonstrate inactivation of p53 by various genetic alterations, such genetic alterations in blood cancers are much less frequent. This raises the question of how these blood cancers develop in the presence of a major guardian. One possibility is that blood cancers have developed alternative ways of inhibition of p53 activity, thus bypassing the requirement for p53 genetic alterations. Indeed, the inhibition of p53 activity through elevation of its negative regulators has been documented in some blood cancers. A direct relationship has been shown between the expression level of a major proliferating protein called nucleophosmin (NPM), and a variety of human cancers, suggesting a potential role of NPM in cancer development. In fact, NPM has been proposed as a cancer marker for leukemia, colon, ovarian, prostate and gastric cancers. We and others have recently identified NPM as a novel inhibitor of p53. We demonstrated that NPM inhibits p53 activity and protects cells from stress-induced cell death. We hypothesize that inhibition of p53 is one mechanism by which NPM promotes cell growth and suppresses cell death and that cancer cells may require increased expression of NPM to keep p53 activity in check and to maintain cell survival and growth. We plan to build on our preliminary characterizations of inhibition of p53 by NPM and NPM alterations during disease progression in Fanconi anemia (FA; a blood disease associated with bone marrow failure and leukemia) to explore the role of p53 inhibition by NPM in leukemic development. The long-term goals of our study are to examine the role of NPM in the selection and progression of human cancers in the context of p53 regulation.

The proposed study will be of fundamental importance in understanding the roles of NPM and p53 in the regulation of the two important cellular processes in cancer biology – life and death of the cell. Because the FA disease is similar to therapy-related blood cancers and because FA is considered as an excellent model of leukemia development, our study will not only lend insights to the process of cancer development in bone marrow failure syndromes such as FA, but also yield valuable information on whether NPM may be therapeutically useful in the prevention and treatment of leukemia and other human cancers.

Albert C. Shaw, M.D., Ph.D.
Yale University
$75,000.00 - ***Type IA Topoisomerases in the Maintenance of Genomic Stability and the Response to DNA Damage***
A universal property of cancer cells is the presence of mutations in DNA. In this regard, mammalian cells have evolved sophisticated mechanisms to detect and repair DNA damage; such pathways appear essential for the prevention of mutations in DNA that may facilitate the development of cancer. We are interested in the function of mammalian type IA topoisomerases in the response to DNA damage. Topoisomerases are proteins that carry out the sequential breakage and rejoining of DNA strands; because of the double-helical structure of DNA and the requirement for DNA strands to separate during the transfer of genetic information, these proteins are crucial to maintaining the fidelity and integrity of the genome (i.e. genomic stability). The type IA topoisomerases in particular have been implicated in the maintenance of genomic stability in bacteria and yeast. Mammals have two type IA proteins, Top3α and Top3β ; in bacteria, yeast, and mammals, these proteins interact with members of the RecQ family of DNA unwinding proteins, or helicases. In particular, Top3α interacts with the RecQ protein deficient in Bloom syndrome, a genetic disorder characterized by genomic instability and predisposition to cancer—particularly leukemias. We propose to elucidate the function of type IA topoisomerases using mouse models in which genes encoding these proteins have been deleted. These mouse models will allow us to establish the function of these proteins in the maintenance of genomic stability and the response to DNA damage – processes that are disrupted in leukemias, lymphomas, and other cancers.

Yanping Zhang, Ph.D.
University of North Carolina
$75,000.00 - ***Targeting NPM by tumor suppressor ARF in leukemia and lymphoma***
Nucleophosmin or NPM (a.k.a. B23, numatrin, NO38) is an abundant nucleolar protein implicated in multiple cellular functions. Clinical study indicated that genetic alterations of NPM arise frequently and specifically in leukemia and lymphoma. About a third of acute myeloid leukemia (AML) has NPM mutation. NPM is also observed in chromosomal translocation. The translocation creates fusion products consisting the N-terminal half of NPM fused with anaplastic lymphoma kinase (NPM-ALK) in anaplastic large cell lymphoma (ALCL), with retinoic acid receptor-α (NPM-RARα) in acute promyelocytic leukemia, and with myeloid leukemia factor (NPM-MLF1) in myelodysplastic syndrome. Both the NPM mutations and the NPM fusions have been shown to cause cancer. It is not clear why these happen or how to control them. We have recently found that the tumor suppressor ARF physically binds NPM and functionally inhibits it. With this finding, it may mean a drug that mimics ARF could help inhibit the NPM protein and cancer cell growth. We propose to investigate the potential of NPM as a molecular target for cancer therapy.

POSTDOCTORAL FELLOWSHIP AWARDS

Stephane Bodin, Ph.D.
University of California
$60,000.00 - *Role of plasma membrane lipids in leukocytes polarization and migration*
Invasion of lymphoma and leukemia cells in the body leads to the spread of metastases, a critical step in the development of cancers. Invasion can be due to defects in the control of cellular migration. An initial step in migration is the stable acquisition of cellular polarity consisting of the formation of a leading-edge moving forward and a retracting rear-edge. In order to design therapeutic strategies to prevent non-controlled cell migration, it is of particular importance to understand in detail the mechanisms regulating cell polarity. The production of two particular lipids called PI(3,4)P2 and PI(3,4,5)P3 exclusively at the leading-edge membrane is known to be critical for the regulation of cellular polarity. Unknown are the mechanisms that restrict the distribution of these lipids to the leading-edge and the exact processes by which they act. In the continuity of my current work, I aim to examine further the spatio-temporal localization of PI(3,4)P2 and PI(3,4,5)P3 and to investigate how the cell membrane organization participate to restrict the distribution of PI(3,4)P2 and PI(3,4,5)P3, and therefore to regulate cell polarity. My second objective is to identify new protein effectors of PI(3,4)P2 and PI(3,4,5)P3, that would represent potential pharmacological targets to prevent invasion of leukemia cells.

Enrique Cepero, Ph.D.
Cold Spring Harbor Laboratory
$60,000.00 - *Regulation of leukemic stem cells by tumor supressors*
Blood cancers and other malignancies are typically caused by mutations that alter genes that normally control cell proliferation and cell survival. Chronic myelogenous leukemia (CML) is initiated by the *bcr-abl* oncogene that is unique in its ability to transform blood cells to a malignant state. Bcr-abl also has an enzymatic activity (known as a kinase) that is important for its leukemia-promoting activity and can be effectively blocked by certain cancer drugs. However, while Bcr-abl inhibitors show remarkable activity against CML they are not curative, perhaps because the Bcr-Abl gene preferentially affects “stem cells” that are capable of continually feeding the leukemia and are more resistant to therapy. The goals of this application are to determine whether mutations in tumor suppressors that contribute to CML progression to “blast crisis”, a more aggressive and drug resistant state, impact stem cell function, survival and self-renewal in cultured cells and in mice. They also will identify novel genes that influence the progression of CML to growth factor receptor signaling that may lead to growth factor independence.

Heather E. Kendall, Ph.D.
University of Vermont
$60,000.00 - *Metabolic polymorphisms as biomarkers for pediatric lymphoid cancers and mutation risk.*
Variations in human genes responsible for detoxifying chemicals in the body can alter the extent of damage to DNA, and effect human cancer risk. The goal of this study is to investigate the prevalence of genetic variations, called polymorphisms, in ten genes in two different groups of children, one with an elevated leukemia and lymphoma risk, and the other with an increase in the occurrence of mutations, or changes in their DNA, in non-tumor cells, following chemotherapy. The first group were shown by the Environmental Protection Agency to have a 70% higher incidence of childhood cancer compared to the statewide incidence, as a consequence of drinking contaminated drinking water from hazardous waste sites in Toms River, NJ from 1979-1995. The second group was shown to have a 30-1300-fold increase in the occurrence of mutations at a marker gene, not involved in cancer, following chemotherapy treatment for leukemia. These studies will provide important information about the usefulness of studying polymorphisms in genes involved in chemical processing as markers of susceptibility both to childhood cancers, and for acquiring mutations in children receiving chemotherapy.

Malay Mandal, Ph.D.
University of Chicago
$60,000.00 – ***Mechanisms of Notch-induced Leukemia***
More than 50% of human T-cell acute lymphoblastic leukemias (T-ALLs) either over-express or harbor activating mutations of the Notch oncogene. Using mouse models of T-ALL and gene profiling of human patient samples, we have shown that induction of T-ALL depends on the functional co-operation of Notch with the Pre-T Cell Receptor (Pre-TCR) signaling cascade, a pathway active during early T cell development. The mechanisms of this co-operation are currently unknown. In this proposal, we attempt to determine the nature or the interaction of the two pathways by directly testing whether pre-TCR expression is a downstream target of Notch or if they are parallel signaling cascades that provide separate (but essential) signaling. Moreover, we determine the outcome of these signaling pathways: the genes that are regulated by either the pre-TCR or Notch and are important for cell transformation. We try to define the essential role of each pathway at a specific stage of the disease progression and whether there are secondary genetic events that are important for the establishment of the disease. Finally, using the novel tool of RNA interference we attempt to inhibit leukemia by targeting genes-targets of the pre-TCR and Notch pathways. Our studies directly address key questions in the understanding of T cell leukemia, and they will identify molecular pathways and gene-targets, essential for induction and / or maintenance of leukemia.

Tiffany Ting-Yi Huang, Ph.D.
Harvard University
$60,000.00 – ***The Role of B7-H4 in peripheral T cell tolerance and regulation***
Our immune system has a very complex and stringent regulation in order to keep the immune cells in check while they protect us from the harmful bacteria and viruses. A list of molecules called 'costimulatory molecules' play important roles in this regulation. B7-H4, one of the newly identified costimulatory molecule, is the interest of this study. With the use of neutralizing antibodies, fusion proteins, and animal models, we will study the characterization and functional analysis of B7-H4 for its role in immune regulation. In addition, we also want to examine the role of B7-H4 in tumor immunology. One melanoma animal model will be used to delineate the potential role of this molecule in tumor therapy.

Hui-I Kao Tom, Ph.D.
The Johns Hopkins University
$60,000.00 - ***Identification and Characterization of a Nuclease Involved in Telomere Processing***
Chromosomes, which contain the entire set of instructions for a cell, consist of linear DNA. These chromosome ends are not completely copied during cell division. Telomeres are the structures at chromosome ends that prevent the loss of genetic information from the ends with each division. Telomerase is an enzyme that allows telomere length maintenance. Telomerase is required for cell viability; short telomeres lead to cell death and chromosome instability. Understanding both telomere and telomerase may provide new targets for potential therapies. In this proposal, we intend to identify a protein that maintains specific structures at the telomere. We will characterize the proteins and investigate the mechanism by which it maintains the telomeric structures. This analysis may lead to a new fundamental understanding of telomere function and may provide new targets for cancer chemotherapy.