

# Kentucky Lung Cancer Research Program

## Cycle 4 Grant Abstracts

<u>Principal Investigator</u>		<u>Grant Research Title</u>
Timothy Aldrich, Ph.D.	UL	Lung cancer in Kentucky environmental/occupational factor
Haribabu Bodduluri, Ph.D.	UL	Role of Chemoattractant Mediated Inflammation in Development and Progression of Lung Cancer
Jason Chesney, M.D., Ph.D.	UL	Glycolysis and Lung Cancer
Donald Cohen, Ph.D.	UK	Deviation of Anti-Tumor Immunity via IL-10 Production in Non-Small Cell Carcinoma
C. Gary Gairola, Ph. D.	UK	Genetic Polymorphism and Lung Cancer: Animal Models of Tobacco Carcinogenesis
Lu-Yuan Lee, Ph.D.	UL	Mechanisms Underlying Pulmonary Stresses in Small Cell Lung Cancer
Robert A. Mitchell, Ph.D.	UK	Promotion of non-small cell lung cancer signaling and development by MIF
Thomas L. Roszman, Ph.D.	UK	Calpain Dependant Lung Cancer Cell Migration and Invasion
Jamie L. Studts, Ph.D.	UL	Behavioral, Cognitive, and Affective Responses to Lung Cancer Screening
John R. Yannelli, Ph.D.	UK	Characterization of two newly described lymphocyte Defined Antigens in NSCLC
H. Sam Zhou, Ph.D.	UL	Destroy Lung Cancer with Combined Effects of Apoptosis and Oncolysis

Principal Investigator: **Timothy Aldrich Ph.D., University of Louisville**

Research Title: Lung cancer in Kentucky environmental/occupational factor

This grant assesses the risk of lung cancer developing in people exposed to hazardous environmental or occupational chemicals. In western Jefferson County, Calvert City, Paducah, and Ashland, Kentucky there are multiple industrial plants that may contribute to the lung cancer risk of their communities. Portions of these communities are economically disadvantaged and/or minority populations that may experience disparities in health due to environmental pollution and cultural-social factors, increasing the local lung cancer risk.

In Kentucky, there are active community-based coalitions for cancer control and environmental concerns. These coalitions represent model opportunities for effective risk communication to the citizens of affected communities. We specifically link occupational and environmental data with lung cancer rates [incidence and mortality] using computerized, geographic software. Next we investigate relationships that may be found between occupational and environmental exposure with selected rare lung cancer outcomes (for example pleural cancer), and among specific sub-populations (e.g., non-smoking women, African-Americans).

As part of this work, we perform statistical analyses to assess the relationship of hazardous exposures with lung cancer outcomes while also assessing the impact from socio-demographic factors [e.g., smoking prevalence rates, reduced health care utilization]. Finally, we will develop model elements for communicating reliable and understandable lung cancer risk information to Kentucky citizens, related to environmental and occupational exposures.

Principal Investigator: **Haribabu Bodduluri, Ph.D., University of Louisville**

Research Title: Role of Chemoattractant Mediated Inflammation in Development and Progression of Lung Cancer

Most lung cancers are associated with smoking, but most smokers do not get lung cancer. In other words, why do certain individuals suffer lung cancer at an early age, after a few years of smoking, while some centenarians tolerate lifelong smoking without dying of the same disorder? One possibility is that chronic inflammation in the lung, caused by smoke inhalation, enhances the ability of carcinogens in smoke to induce cancer. If so, then people who have chronic inflammation of the lung tissues may be at greater risk for developing cigarette smoke-associated lung cancer.

Over one hundred years ago, physicians observed that many cancers arise from sites of infection, chronic irritation, and inflammation. This is particularly apparent in the lung; an organ constantly exposed to a variety of environmental insults. However, the exact nature of the relationship between inflammation and lung cancer remains unknown.

The overall goal of this project is to examine the idea that the degree of chronic inflammation induced in an individual by an irritant strongly increases the potential of cancer-causing chemicals (carcinogens) to actually induce tumors. We propose to carry out these studies using genetically engineered mice that do not mount inflammatory reactions or do not mutate DNA after they have been exposed to carcinogens. We plan to examine the number of lung tumors that arise in these mice after exposure to a smoke-associated carcinogen, with or without exposure to another chemical that causes lung inflammation. We will also characterize genetic alterations induced in the lung cells of these mice using contemporary molecular biological techniques. This project addresses fundamental questions in tumor biology and may suggest potential strategies to reduce the incidence of lung cancer in exposed individuals.

Principal Investigator: **Jason Chesney, Ph.D., University of Louisville**

Research Title: Glycolysis and Lung Cancer

Lung cancer needs the sugar glucose to grow more than normal, healthy tissues. The best test for identifying and monitoring lung cancer, called PET, relies on this distinct characteristic. Glucose is degraded by lung cancers to lactic acid, and this can cause a life-threatening condition called lactic acidosis. While it is well known that lung cancer uses glucose to grow more than normal tissues, the specific mechanism that causes this effect is not known.

If we can determine how lung cancer uses high glucose, then we can develop drugs that can stop the high glucose use, and stop the lung cancer from growing. We have identified an enzyme that may allow lung cancers to increase their use of glucose – iPFK-2. We have shown that this enzyme is over-produced by lung cancers and can increase glucose use in leukemia. *Our hypothesis is that iPFK-2 is needed by lung cancers to use abnormally high amounts of glucose.*

Since we can develop drugs that inhibit iPFK-2 and possibly the growth of lung tumors, we want to know if lung cancers need iPFK-2 for glucose use. Our specific plans are to over-produce or delete the iPFK-2 gene in lung cancer cells and examine the effects on the ability of the cells to use glucose and to grow like a cancer. We predict that without iPFK-2 the lung cancer will not be able to use glucose and will not be able to grow. Next, we will identify drugs that might inhibit iPFK-2 and test their ability to inhibit both iPFK-2 and tumor growth in mice. Our long-term goal is to develop new drugs that target the unique ability of lung cancer to use high amounts of glucose.

Principal Investigator: **Donald Cohen, Ph.D., University of Kentucky**

Research Title: Deviation of Anti-Tumor Immunity via IL-10 Production in Non-Small Cell Carcinoma

Lung cancer kills more individuals in Kentucky each year than anywhere else in the U.S. Whether a person survives lung cancer depends on a number of factors; however, the ability of a patient's immune system to attack his/her lung cancer is one important dynamic. Many lung cancer cells produce a hormone-like molecule called interleukin-10 (IL-10). This molecule will suppress many aspects of the immune system, but its role in the development of lung cancer is virtually unknown.

We have developed a model in which mouse lung-cancer cells express different levels of IL-10 and grow to form tumors when injected into mice. Using this model we have demonstrated that IL-10 alters the immune cell types and their state of activation within the tumor. We propose to use this model to determine if production of IL-10 by lung cancer cells suppresses the ability of mice to develop an effective immune response against their tumors and whether IL-10 expression allows lung tumors to grow faster in mice and to spread more effectively to other tissues in the body. If the release of IL-10 by lung cancer cells inhibits the immune response against lung cancer cells; and enhances the growth and spread of the tumor in mice; then it may suggest that therapies to control the production of IL-10 in lung cancer. These therapies might enhance a patient's ability to generate an immune response against their own tumor and subsequently increase the chances for survival for lung cancer patients.

A final goal of this study is to neutralize IL-10 production by lung tumor cells *in vivo* to determine if a more effective anti-tumor response can be elicited in tumor-bearing mice. These studies will provide a framework for a future NIH grant application to determine if the poorer prognosis of lung cancer patients with IL-10-producing tumors is due to alterations in the immune response against their tumors. Findings from these studies will allow us to determine if investigations into therapies that control IL-10 synthesis by lung cancers should be pursued in future studies. Finally, these studies will enable us to establish new interactions with other tumor immunologists and tumor biologists within the state of Kentucky and initiate the intellectual and scientific interactions that are necessary for development of successful NIH program project applications.

Principal Investigator: **C. Gary Gairola, Ph.D., University of Kentucky**

Research Title: Genetic Polymorphism and Lung Cancer: Animal Models of Tobacco Carcinogenesis

Smoking as a major risk factor for lung cancer, is the leading cause of cancer-related death in Kentucky and in the United States. In spite of extensive research, the underlying mechanisms of how smoking causes cancer are poorly understood. Studies to identify chemo preventive agents that will protect against smoking-mediated cancer have been unsuccessful. One of the reasons for this is a lack of a suitable animal model in which lung cancer can be produced experimentally by inhalation exposure to cigarette smoke.

We hypothesize that since smokers who develop cancers have been found to lack or possess defective forms of either cancer promoting genes or cancer protective genes, it is necessary to employ test animals that

In this study we will test genetically modified mice lacking specific detoxification enzyme genes for their susceptibility to cigarette smoke-induced tumorigenesis. Our major aim is to establish a suitable animal model that can be used (1) to examine the mechanisms of tobacco carcinogenesis, and (2) for identification of chemo preventive agents that will protect against tobacco smoke carcinogenesis. It is expected that these studies will not only provide a suitable animal model but will also help identify target genes of smoke carcinogens.

Principal Investigator: **Lu-Yuan Lee, Ph.D., University of Kentucky**

Research Title: Mechanisms Underlying Pulmonary Stresses in Small Cell Lung Cancer

Small cell lung carcinoma accounts for about twenty percent of all lung cancers and kills approximately thirty thousand patients in this country every year. Patients with primary small cell lung cancer suffer from persistent cough, dyspnea and lingering chest pain. These pulmonary stresses severely effect a patient's quality of life. The long-term goals of this research project are to uncover the mechanisms underlying these symptoms, to delay the deteriorating process of the disease, and to develop new therapeutic strategies for alleviating these symptoms.

One of the characteristic features of these cancer cells is their ability to secrete a variety of chemical substances, and many of these substances have distinct biological activities. The working hypothesis of this study is that the gastrin-releasing peptide, a major type of these chemical substances, stimulates a specific group of sensory nerves in the lungs that are particularly sensitive to chemical irritants. These nerves, when activated, not only can cause chest pain, dyspnea and cough, but also release certain neurochemicals that can in turn promote the tumor growth. Therefore, an interaction between these sensory nerves and cancer cells in the lungs may play a critical part in the deteriorating process of the disease.

The main objective of this study is to uncover the mechanism underlying the action of the gastrin-releasing peptide on the sensory nerves of the lung. We will isolate and culture the cells of these sensory nerves from experimental animals (rats), and then expose the nerve cells to the gastrin-releasing peptide. Data obtained from our pilot experiment have already demonstrated the feasibility and potential significance of this study. We believe that the results obtained from this study will improve our understanding of the pathogenic role of the interaction between these sensory nerves and the cancer cells. Further, the new information should help to develop new therapeutic strategies for reducing the respiratory stresses in patients suffering from this type of lung cancer.

Principal Investigator: **Robert A. Mitchell, Ph.D., University of Louisville**

Research Title: Promotion of non-small cell lung cancer signaling and development by MIF

Lung cancer is the leading cause of cancer deaths worldwide, with over 150,000 deaths predicted for 2003 in the United States alone. For treatment purposes, lung cancer is divided into two histopathologic classes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which differ in their responses to therapy. Approximately 80% of lung cancer cases are classified as NSCLC, while SCLC accounts for ~18%.

These two classes are characterized by distinct patterns of oncogene activation, tumor suppressor gene mutation, and chromosomal alterations. Specifically, mutations in the retinoblastoma (Rb) tumor suppressor gene are common in SCLC while NSCLC rarely acquire Rb mutations. As retinoblastoma inactivation is a functional requirement for lung cancer progression, the identification of endogenous cellular mediators of Rb inactivation in NSCLC is essential. We have recently identified that a secreted pro-inflammatory growth factor, migration inhibitory factor (MIF), functionally activates pathways that lead to Rb inactivation. Moreover, mesenchymal cells (*i.e.* cells *not* of an epithelial origin) lacking MIF are resistant to malignant transformation induced by NSCLC-associated oncogenes and this resistance is due to inefficient Rb inactivation.

However, more studies are required to investigate fully the *in vitro* and *in vivo* requirements for MIF in epithelial cell derived non-small cell lung cancers. Experiments planned in this project seek to elucidate the contribution, influence, and mechanisms of MIF-dependent modulation of NSCLC Rb inactivation, epithelial cell malignant transformation, and *in vivo* lung tumorigenesis. It is hoped that results from these studies will provide evidence of an important new target for future lung cancer chemotherapeutic strategies.

Principal Investigator: **Thomas L. Roszman, Ph.D., University of Kentucky**

Research Title: Calpain Dependant Lung Cancer Cell Migration and Invasion

The purpose of this research is to investigate the mechanisms involved in the migration and metastasis of lung tumor cells. The hypothesis to be tested is that the action of a protease termed calpain (Cp) is integral to lung cancer cell migration, invasion, and metastasis. Proteases are enzymes that cleave proteins and, therefore, are critical for the regulation of cell function. Cp and its regulatory molecules, calpastatin (Cs) and a regulatory subunit (collectively referred to as the Cp/Cs network) participate in a number of cellular functions including the regulation of normal and malignant cell movement.

The migration and invasion of tumor cells into adjacent tissue sites or the metastasis to distant sites, such as lymph nodes, in part is modulated by receptors on these cells, which bind to their ligands on the extracellular matrix (ECM). The experiments planned in our first aim of this grant examines the role of the Cp/Cs network in assays designed to quantitate the adhesion and migration of lung cancer cells to the ECM. Thus, one component of our first aim is to investigate the role of the Cp/Cs network in the adhesion and migration of a lung cancer cell line (A-549) on the ECM protein laminin.

In addition, we will assess the role of the Cp/Cs network on the ability of this lung cancer cell line to invade into the ECM. Results from these studies will establish the importance of the Cp/Cs network in lung cancer cell migration and metastasis and pave the way for the second aim of this grant. This aim is designed to investigate mechanistically how the Cp/Cs network can regulate the adhesion, shape change, and migration of A-549 binding to the ECM. The hypothesis to be tested is that the Cp/Cs network translocates to subcellular organelles in the A-549 cells and this is required for regulation of cell migration and metastasis.

These studies use microscopic and biophysical techniques to probe for the intracellular locations of Cp and its regulatory proteins in A-549. Experiments also determine how Cp and its regulatory proteins bind to these locations and what proteins are cleaved by Cp. The collective results from these studies will prove useful in developing new therapeutic strategies to prevent the growth and spread of lung cancer.

Principal Investigator: **Jamie L. Studts, Ph.D., University of Louisville**

Research Title: Behavioral, Cognitive, and Affective Responses to Lung Cancer Screening

Lung cancer is a devastating disease and is the leading cause of cancer-related death in the United States. Approximately 171,900 individuals will be diagnosed with lung cancer in 2003 and 157,200 Americans are expected to die from the disease. However, data suggest that when detected early, individuals diagnosed with lung cancer are significantly more likely to survive the disease. The combination of high mortality and more effective early stage treatments creates a context where lung cancer screening programs could significantly reduce the impact of the disease. Unfortunately, efforts to develop effective lung cancer screening have been largely unsuccessful and are likely to be associated with more risks than benefits.

However, recent advances in spiral-computed tomography (spiral CT) have created renewed interest in lung cancer screening. Early results of spiral CT-based screening suggest several potential benefits associated with this method of lung cancer screening. Interestingly, one preliminary behavioral study conducted with spiral CT screening data suggested that significant numbers of lung cancer screening participants attempt to quit smoking following screening. Approximately 23% of smokers quit smoking and another 26% reduced smoking during the screening trial. Although data optimistically suggest that participating in screening may promote smoking cessation and potentially reduce an individual's risk of developing lung cancer, additional studies investigating behavioral outcomes following spiral CT are necessary.

The purpose of this study is to examine a broader set of behavioral and psychological responses to lung cancer screening. Participants will be drawn from a randomized trial of lung cancer screening being conducted at Jewish Hospital and the University of Louisville. This study will provide the first longitudinal data from a randomized trial regarding behavioral responses to lung cancer screening. This study will extend previous research by examining other important psychological responses to screening and by studying how sociodemographic variables, psychological responses, and screening results might relate to self-initiated smoking cessation. Additionally, this study will provide the first data using biochemical methods (i.e., cotinine) to verify self-reported smoking changes in lung cancer screening participants.

Principal Investigator: **John R. Yannelli, Ph.D., University of Kentucky**

Research Title: Characterization of two newly described lymphocyte Defined Antigens in NSCLC

Non-small cell lung cancer (NSCLC) is a leading killer of men and women around the world and is responsible for many deaths in the Commonwealth of Kentucky. While surgery, chemotherapy and/or radiation therapy remain the key treatment options, our lab is investigating immunotherapy as a 4<sup>th</sup> modality of treatment for residual disease.

Immunotherapy is being investigated world wide as an additional therapy for cancer. It relies on the body's ability to recognize and destroy cancer using the immune system. While it was believed that the immune system from cancer patients is defective, we now know that the immune system remains intact. However, tumor specific suppression of certain aspects of the immune response can occur, particularly those against the patient's tumor.

Immunotherapy attempts to reduce that suppression and through *in vitro and in vivo* means, re-target the patient's immune system to destroy their cancer. We currently are immunizing NSCLC patients with dendritic cells (DCs), a potent immune activating cell, with antigens derived from lung cancer cells and have demonstrated a positive immunologic response to the antigens contained in the vaccine in our trial.

In the current project, we seek to characterize two novel-antigens we have identified on NSCLC tumor cells that serve as targets for immune recognition. The genes, myo-inositol monophosphatase (IMPA) and guanine nucleotide binding protein (GNAS) were identified using a method we developed in our lab. We want to characterize these antigens and determine their overall relevancy to NSCLC. If our studies show us that they are restricted to tumor tissue and are expressed on the majority of lung cancer tumor cells, these antigens will be prime candidates for future immunization strategies.

Our ultimate goal is the development of a vaccine which can be utilized not only in the setting of complementing existing conventional therapies but we hope to have a vaccine which might prevent lung cancer in susceptible populations across Kentucky.

Principal Investigators: **H. Sam Zhou, Ph.D., University of Louisville**

Research Title: Destroy Lung Cancer with Combined Effects of Apoptosis and Oncolysis

Adenoviruses have evolved strategies to induce quiescent cell into S phase and to inhibit apoptosis to prevent premature cell death for productive replication. These two processes, inducing cell proliferation and inhibiting apoptosis, are primarily controlled by adenovirus E1A (induce growth) and E1B (inhibit apoptosis). There are significant similarities between cancer cells and adenovirus-infected cells in terms of boosting cellular proliferation and inhibition of apoptosis. Proliferation and apoptosis are well balanced and closely controlled in normal cells, while disrupted in all cancer cells. In our preliminary studies, we have showed that a mutant adenovirus selectively replicated in cancer cells and destroyed tumor in vivo (manuscript accepted by Cancer Research). We also showed that Adhz60 with deletion of entire E1b can induce apoptosis in cancer cells, cells that resist to apoptosis allow Adhz60 to replicate, eventually all cancer cells were destroyed. In contrast, normal cells do not support the E1b deleted virus replication (submitted to Nature Biotechnology). Based on these findings, we are proposing to study the interactions of cellular E2F-1 with viral E1A and E1B proteins, to study the effects of virus infection on apoptosis and virus replication. We hypothesize that host cellular factors, which deregulate proliferation and inhibit apoptosis in cancer cells, may replace these functions encoded by the adenoviral E1 genes. Therefore, viruses with mutated E1 genes could selectively replicate in cancer cells but not in normal cells. Specific aims: 1) Investigate effects of E1A levels on cell apoptosis and virus replication in cancer cells; 2) Enhance virus selective replication in lung cancer cells with E2F-1 gene. Based on the better understanding of the mechanisms involved, we will be able to design and to develop further strategies that will enhance cancer cell apoptosis and cytolysis, and can be utilized eventually in patients. The investigators participating in this application have very broad backgrounds ranging from virology to molecular biology to the clinical care of patients with cancer. This breadth of experience and knowledge will benefit our efforts to determine the mechanism of viral replication in cancer cells and to create more specific and efficient agents for such therapeutic approaches. We are quite optimistic that the promising preliminary data that we have generated predicts that this will be a very important new approach for the treatment of cancer.