

Kentucky Lung Cancer Research Program

Cycle 2 Grant Abstracts

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Principal Investigator: **Bradley Anderson, Ph.D., University of Kentucky**

Research Title: Anti-topoisomerase I aerosols for lung cancer therapy

The plant alkaloid camptothecin (CPT) has shown significant anti-topoisomerase I and antitumor activity against a wide variety of human tumors xenografted in nude mice. In recent studies conducted by Dr. Knight, professor and chair of the Department of Physiology of the Baylor University College of Medicine, the administration of dilauroylphosphatidylcholine (DLPC) liposome aerosols containing 9-nitrocamptothecin (9-NC) have been shown to very effectively inhibit the growth of human breast, colon, and lung cancer xenografts. However, recent results for the laboratory of Dr. Burke at the University of Kentucky presented at the 2001 AACR and ASCO meetings indicate that translation of this very promising aerosol therapy to the clinic will be very difficult to achieve. Clinically relevant camptothecins such as 9-NC contain a lactone ring pharmacophore which is essential for activity, but these drugs hydrolyze rapidly in human tissue and blood. Human serum albumin (HAS) avidly binds the ring-opened form of 9-NC and shifts the equilibrium such that virtually all of the active form of 9-NC disappears in human blood and tissue. This effect is very specific for human serum albumin, with the active lactone levels of 9-NC being some 100-times higher in mouse blood versus human blood which provided a basis for the favorable therapeutic outcomes obtained in the xenograft models in nude mice. Thus, a real need exists for the development of camptothecins that exhibit high lactone stability in human blood tissues. Toward this end, the Burke and Curran labs of the University of Kentucky and University of Pittsburgh, respectively, have developed novel, potent, and highly tissue-stable camptothecins known as silatecans and homosilatecans. DB-67 is the lead silatecan, which has been shown to cure effectively mice of intracranial U87 human glioma xenografts. Through the RAID Program of the National Cancer Institute, the Burke lab is currently generating the necessary data that will fulfill the requirements for a FDA Investigational New Drug application for DB-67 by the end of 2001. These efforts include GMP scale up of DB-67, formulation production, and FDA IND directed toxicology and pharmacology studies.

In the current proposal, we intend to create aerosol formulations of our most promising silatecan and homosilatecan agents. We intend to work with Dr. Chen to optimize this important new anti-topoisomerase I therapy for lung cancer. The pharmacokinetics and efficacy of inhaled silatecans and homosilatecans formulated in DLPC liposomes will be evaluated. C57BL/6 mice with subcutaneous Lewis lung carcinoma, Swiss nu/nu mice with human lung carcinoma xenografts will be studied.

Principal Investigator: **Steven R. Ellis, Ph.D., University of Louisville**

Research Title: LAMR1, a potential lung cancer tumor suppressor gene in chromosomal region 3p21.3

Loss of heterozygosity (LOH) in chromosomal region 3p21.3 is frequently observed in lung cancer. Allele loss in 3p21.3 is induced by tobacco carcinogens and is observed in premalignant lesions of the lung, demonstrating that LOH in 3p21.3 plays a critical role in the early stages of lung cancer. The association of LOH in 3p21.3 with lung cancer points to the presence of tumor suppressor genes (TSGs) in this region. Despite intensive effort, TSGs in 3p21.3 have been difficult to identify because they do not conform to the classical genetic criterion for defining a TSG. Instead, 3p21.3 appears to harbor one or more non-classical TSGs. 3p21.3 appears to contain TSGs that predispose to cancer in a hemizygous state.

The hypothesis to be tested here is that the LAMR1 gene is such a non-classical TSG. LAMR1 encodes a protein, p40, of the small ribosomal subunit. Reduced expression of the *Drosophila* p40 protein leads to hyperplasia in certain tissues. The p40 protein appears to function by regulating alternative forms of translational initiation. Decreasing the level of p40 protein may therefore favor the translation of proteins initiated by these alternative mechanisms. Since many of the proteins initiated by these mechanisms regulate cell growth and division, decreased expression of p40 is further hypothesized to interfere with normal growth controls and promote carcinogenesis.

The specific aims of this proposal are to:

- a. create a mouse ES cell line hemizygous at the LAMR1 locus,
- b. determine the effect of hemizygosity at the LAMR1 locus on translational initiation in ES cells,
- c. create a strain of mice hemizygous at the LAMR1 locus,
- d. compare the frequency of spontaneous tumors in LAMR1 hemizygous mice with wild-type,
- e. determine if hemizygosity at the LAMR1 locus influences spontaneous or chemically induced tumorigenesis in a mouse strain predisposed to lung cancer.

Principal Investigator: **Edward A. Hirschowitz, M.D., University of Kentucky**

Research Title: Detection of Tumor Markers in Peripheral Blood of Non-small Cell Lung Cancer Patients

The insidious nature of lung cancer dictates that <70% of individuals diagnosed are at an advanced stage and thus unresectable. Survival advantages of early stage disease provide the rationale for early detection as a means of reducing lung cancer mortality. Newer and established imaging techniques and strategies for the screening, diagnosis, staging, and management of lung cancer are being widely investigated to improve outcomes and management of NSCLC. Tumor markers, measured in peripheral blood, could similarly impact lung cancer. The sensitivity and specificity of available lung cancer markers limits their clinical application as independent or even complementary tests. The current proposal describes how tumor specific auto-antibodies in NSCLC patient sera can be used to identify tumor antigens from a NSCLC cDNA library and how in turn, these tumor proteins can be used to detect tumor-specific antibody responses in patient serum that can serve as markers for disease. Because no single antibody response is likely to be a comprehensive marker, we propose to use a panel of phages that express a variety of tumor antigens as a serologic test for screening, diagnosis, and management of NSCLC. Fluorescent microarray technology, applied generally to gene discovery, is ideal for this purpose. Preliminary experiments have already identified several NSCLC antigens and show the corresponding autoantibodies are found in cancer patient serum and not in normal serum. These data shows feasibility and proof of concept that supports the rationale behind this proposal.

Principal Investigator: **David M. Kaetzel, Ph.D., University of Kentucky**

Research Title: PDGF and novel gene therapy approaches to lung cancer treatment

The platelet-derived growth factor (PDGF) polypeptides are potent mitogens for connective tissue cells, and their roles in promoting cellular growth and malignant progression have been firmly established for such human cancers as glioblastoma and osteosarcoma. However, the role of PDGF has not been studied as extensively in lung cancer, despite reports of PDGF over expression in all forms of this disease. Overproduction of PDGF by the primary lung carcinoma cell is almost certainly a key factor in various aspects of tumors stromal development (paracrine growth stimulation), while additional evidence has been put forth to suggest that PDGF autocrine growth loops may also be operative.

Our previous research on PDGF has indicated two promising avenues for gene therapy of lung cancer. First, we have developed dominant-negative forms of PDGF that reverse the transformed phenotype in human glioblastoma cells. In this proposed study, we will measure the extent to which these (and other mutant proteins designed to disrupt PDGF-stimulated growth) can block the malignant growth of human lung carcinoma cells, both in cell culture and in vivo mode systems. Second, we have recently identified a DNA enhancer element (ACE66) in the far upstream region of the PDGF-A gene that is highly activated in many human tumor cell lines. In this study, we will employ the ACE66 element to drive expression of the “suicide gene” encoding thymidine kinase and will measure its efficacy for killing lung carcinoma cells in cell culture and in vivo model systems. Recent studies in our laboratory have demonstrated that three tandem copies of the ACE element confer over 30-fold enhancement of transcription in the non-small cell lung carcinoma cell line, A549. Considering the current paucity of transcriptional elements available for directing lung cancer-specific, high-level expression of suicide genes, these proposed studies appear to hold considerable promise for this approach to lung cancer treatment. Overall, the proposed studies should also advance our current knowledge about the specific role play be PDGF in lung cancer development and progression.

Principal Investigator: Carolyn M. Klinge, Ph.D., University of Louisville

Research Title: Interaction of carcinogens with estrogen receptor beta signaling in lung cancer

While the number of women dying as a result of metastatic breast and colon cancer are declining, the mortality associated with lung and bronchus cancer continues to rise. The death rate for female lung cancer exceeds that of breast cancer. Cigarette smoking is an established risk for developing lung cancer, accounting for 17% of female deaths in the U.S. Constituents of cigarette smoke include benz[a]pyrene (B[a]P) and other polycyclic aromatic hydrocarbons (PAHs) are not only direct carcinogens by forming DNA adducts but act as antagonists to estrogen action through their binding to the arylhydrocarbon receptor (AHR).

Historically, lung was not considered an estrogen target tissue, but recent studies have shown that assumption to be false. Most biological responses to estrogens are mediated by two estrogen receptors (ER)--ER α and ER β . ER β has been shown to be expressed in mouse, rat, and human lung. ER β is also expressed in adenocarcinomas of the lung. The biological role of ER β in lung is unknown, but in other tissues, ER β is antiproliferative. Some studies show ER α is expressed in lung, while others do not. We will evaluate the mRNA and protein expression of ER α and ER β in normal bronchial epithelial cells and in non-small cell lung carcinoma cell lines. We have shown that B[a]P-occupied AHR inhibits the activity of ER α and ER β in human breast cancer cells, but whether this is true in lung cells and whether such "cross talk" plays a role in lung carcinogenesis or progression is unknown. In addition to PAHs, cigarette smoke contains heavy metals including cadmium and arsenic that play roles in lung carcinogenesis. Cadmium and arsenic activate ER α , but their effect on ER β is unknown.

The overall goals of this project are to test the hypotheses that 1) B[a]P increases ER β expression, but inhibits ER β activity; 2) cadmium and arsenate inhibit ER β expression and activity, and 3) resveratrol exhibits ER β agonist and AHR antagonist activity in lung cells. The last goal is discovery driven: to identify E2-stimulated target genes in lung cells whose transcription is altered by B[a]P. Identification of estrogen-regulated genes targeted by B[a]P is critical to elucidate the short- and long- term effects of B[a]P on ER β -expressing lung cells and their role in carcinogenesis.

Principal Investigator: **Heinz Kohler, M.D., Ph.D., University of Kentucky**

Research Title: Second-Generation Antibody against HER-2/neu: Preclinical Studies on Lung Cancer Cells

In response to the Kentucky Lung Cancer Research initiative, we propose to produce a second-generation antibody targeting the HER-2 gene product on lung tumors expressing the HER-2/neu growth factor receptor. This proposal is based on previous work in which we used an autophilic peptide to convert monovalent antibodies into polyvalent molecules with enhanced binding and biological properties.

Currently there are several clinical trials ongoing with Herceptin in lung cancer patients. Because Herceptin provides a unique therapy window for lung cancer patients, improving the therapeutic efficacy could generate significant benefits with regard to reducing the therapeutic dose, increasing the synergy with chemotherapy and extending the therapeutic window to tumors with lower expression of the HER-2/neu receptor. This goal will be achieved by increasing the avidity of Trastuzumab through the use of an autophilic peptide. In the specific aims, we will generate an autophilic Trastuzumab antibody against HER-2 gene product and demonstrate improved tumor targeting, test for enhanced ADCC and CDC, cell cycle inhibition and apoptosis, and inhibition of cell growth. These pilot studies will set the stage to evaluate the autophilic modification of Herceptin in future clinical studies.

Principal Investigator: **Guo-Min Li, Ph.D., University of Kentucky**

Research Title: Alterations of DNA mismatch repair genes in lung cancer

Lung cancer is the most lethal cancer in the United States and Kentucky is the leading state for lung cancer death in the nation. However, the molecular basis of the deadly disease is still unknown. Previous studies have shown that lung cancer is often associated with deletion of chromosome 3p, where a mismatch repair (MMR) gene, *MLH1*, is located. Recently, a substantial fraction of lung cancer have been shown to manifest frequent alternations in simple repetitive sequences (also called microsatellite instability), a phenotype that was initially identified in hereditary non-polyposis colorectal cancer and was caused by loss of function in a genome maintenance pathway called MMR. These findings strongly suggest a close association of lung cancer with MMR defects.

To test this hypothesis, this study will utilize both genetic and biochemical approaches to analyze relationship between lung cancer and MMR deficiency. First, lung tumors will be screened for important MMR genes, *MSH2* and *MLH1*, by immunohistochemistry. Specific alternations/mutations will be determined in tumors lacking expression of *MSH2* and *MLH1* using the combined technology of PCR, single strand conformation polymorphism, and DNA sequencing analysis.

Second, individual alternations identified will be introduced into the *MSH2* or *MLH1* baculoviral clones by site-directed mutagenesis. To determine actual impact of these alterations on lung carcinogenesis and MMR function, the mutant baculoviral recombinant proteins will be examined for their ability to restore MMR to known *MSH2* or *MLH1* mutant extracts using an in vitro functional assay.

Finally, tumors lacking *MLH1* Expression will be analyzed for hypermethylation of the *MLH1* promoter, whose methylation has been shown to silence transcriptionally the *MLH1* expression in other cancers. Based on our preliminary studies, we believe that both deletions and point mutations of MMR genes will be identified in lung cancers, and this study will provide useful information for lung cancer etiology, early detection, and treatment.

Principal Investigator: **Thomas C. Mitchell, Ph.D., University of Louisville**

Research Title: T cell function in nicotine-treated mice

Nicotine has anti-inflammatory properties that may impair T cell-dependent immunity in long-time smokers. Heavy smokers who are pharmacologically immunosuppressed after cardiac transplant have rates of lung carcinogenesis that far exceed those associated with smoking alone, indicating that immune responses normally slow or prevent the development of lung cancer. Hence, nicotine may contribute to carcinogenesis by impairing the normally protective actions of tumor-specific T cells.

Inflammation is needed to establish productive T cell responses to infectious pathogens and presumably to tumor antigens. For example, immunizations performed with purified antigen alone, in the absence of a pro-inflammatory adjuvant, deplete antigen-responsive T cells from circulation because the T cells begin to proliferate but go on to die. Immunological adjuvants therefore boost immune responses in part by increasing the survival of T cells during or after they have been activated by antigen. In our preliminary experiments, the combined treatment of mice with nicotine plus adjuvants greatly potentiated the adjuvant-induced survival effect. This observation agrees with reports in the literature that acute doses of tobacco or nicotine boost immune responses temporarily.

We will begin to analyze the potential contribution of nicotine to carcinogenesis by first understanding the acute effects of nicotine on T cell function and survival. This will be done by testing for 1) the acute effects of nicotine on T cell survival and function, 2) the identity of the nicotine receptor subset that transduces survival signals in T cells, and 3) the identity of anti-apoptotic factors induced in T cells by exposure to nicotine. Establishing which pathways are affected by short-term exposure to nicotine will then set the stage for identification of pathways that may become de-sensitized after chronic exposure to nicotine. Understanding how tumor surveillance is inhibited by nicotine will improve immunotherapies intended to treat current smokers as well as former smokers who use tobacco-free nicotine to decrease their abuse of tobacco.

Principal Investigator: **Jeffrey A. Moscow, M.D., University of Kentucky**

Research Title: Drug transport genes as novel targets in lung cancer

Intracellular drug accumulation is a critical determinant of cellular sensitivity to anticancer agents, and intracellular drug accumulation is regulated by membrane-associated drug transporters. Therefore, we hypothesize that drug transporters themselves can be considered as targets for lung cancer therapy, by identifying both the transporters highly expressed in lung cancer and the anticancer drugs that are substrates for those transporters. Genomic data has revealed dozens of drug transport genes, each of which might contribute to the uptake of anticancer drugs. To begin to target these transporters for lung cancer therapy, we have measured the RNA levels of over 20 OAT, organic cation transporter (OCT), and nucleoside/nucleobase transporter genes in five lung cancer cell lines. Preliminary data from these studies demonstrate some of these drug transport genes are over-expressed in lung cancer cell lines, making these transport genes potential targets for novel lung cancer therapy. This is significant since we have found that a transport gene over-expressed in leukemia cell lines is also over-expressed in leukemic blasts from patients. Preliminary data has also demonstrated that we can successfully identify anticancer drugs that are substrates for transport genes. The specific aim of this study is to apply this paradigm to the potential therapy of lung cancer, by identifying drug transport genes highly expressed in lung tumors, and to identify anticancer drug substrates for these transporters. This will be accomplished by measuring RNA levels of approximately 40 transport genes in lung tumors, and to then to use gene transfection studies to confirm functional relationships between anticancer drugs and transporters over-expressed in lung cancer. These studies will give us the necessary preliminary data we need to focus future grant applications on specific proposals for novel therapeutic approaches for lung.

Principal Investigator: **Steven Myers, Ph.D., University of Louisville**

Research Title: Biomarkers of maternal and fetal tobacco smoke exposure

Women who smoke during pregnancy expose themselves as well as their developing fetus to numerous tobacco smoke carcinogens, many of which may be harmful to the fetus. Although much data concerning the effects of smoking is in fact known, few studies have carried out detailed investigations as to the effect of tobacco smoke on the developing fetus. In order to extend investigations into the effects of tobacco smoke on the fetus as well as to develop and validate biomarkers of tobacco smoke exposure in newborns, we will undertake a series of investigations to test the following hypotheses: (1) that the formation of fetal hemoglobin (Hb) tobacco smoke carcinogen adducts is proportional to maternal smoking as well as maternal Hb adducts; (2) that the formation of maternal and fetal Hb adducts are dependent on maternal and fetal genotype to glutathione S-transferase M1 and T1 (GSTM1, GSTT1), N-acetyltransferase (NAT1 and NAT2) and CYP1A1 and (3) that there is a correlation between the formation of DNA adducts and maternal and fetal Hb adducts. To test these hypotheses we have proposed specific aims which include: (1) Recruitment of 500 smokers and nonsmokers into the study to undertake an investigation of Hb — carcinogen adducts as well as correlations of these adducts with assessed genotypes; (2) Characterization of both maternal and fetal Hb for the tobacco smoke carcinogen adducts 4-aminobiphenyl benzo(a)pyrene, and the tobacco specific nitrosamines NNN and NNK; (3) Determination of the influence of pharmacogenetics on maternal and fetal Hb adduct levels; and (4) Characterization of DNA adducts and DNA repair efficiency in lymphocytes and the relationship to Hb adducts, genotype, and smoking histories.. This research will further clarify the use of Hb as a biomarker of tobacco smoke exposure and will also emphasize the health significance of maternal smoking during pregnancy and its effects on the fetus.

Principal Investigator: **Daniel J. Noonan, Ph.D., University of Kentucky**

Research Title: Targeting Retinoic Acid Treatment of Lung Cancers

Vitamin A derived retinoic acid, along with its intracellular receptors, have been linked to the pathology of lung cancer for over 2 decades. Although preclinical and epidemiological results with retinoids have generated mixed results, more recent studies utilizing synthetic analogs of retinoic acid that target specific intracellular receptors for retinoic acid, have generated renewed interest in use of retinoids as therapeutic agents for the treatment of lung cancer. A molecular mechanism for the activities of retinoic acid has recently been defined. Unfortunately the complexity of this mechanism, its ability to act upon any or all of 6 different intracellular receptors which in turn can be modulated by a variety of tissue-specific co regulatory molecules, suggest that establishment of a molecular mechanism for retinoids in lung will be fundamental to defining safe and efficacious retinoid therapeutics for the treatment of lung cancer. Utilizing a retinoid based functional cloning strategy we have identified a human homolog of the mouse neural cell proliferation and differentiation (NPDC) gene that appears to be highly expressed in normal lung but minimally expressed if not absent in a limited number of lung tumors we have examined. The potential significance of this observation resides in previous studies with the NPDC protein that demonstrate retinoic acid can upregulate its expression and that expression of NPDC can suppress cellular proliferation and transformed phenotypes. These data, coupled with many previous studies correlating loss of retinoic acid receptor expression and retinoid sensitivity in lung cancers, form the foundation of our hypothesis that loss of regulation of retinoid expression of NPDC can contribute to the pathology of lung cancers. To examine this hypothesis, two specific aims are proposed in this grant. 1. To investigate correlations between NPDC and retinoid receptor expression during lung carcinogenesis we will examine tumor and normal human lung specimens for their expression of NPDC and retinoic acid receptor genes. 2. To investigate retinoid regulation of NPDC expression, we will molecularly dissect the promoter of the human NPDC gene for its ability to bind retinoic acid receptors and serve as a target for regulation by therapeutic retinoids. The potential diagnostic and therapeutic information generated by these data could directly impact the early detection and epidemiology of lung cancer as well as advance the effectiveness and/or understanding of current strategies for treatment of lung cancer.

Principal Investigator: **John O. Trent, Ph.D., University of Louisville**

Research Title: Design of Nucleolin Inhibitors

We have discovered antiproliferative guanine-rich oligonucleotides (GROs) that form stable quadruplex structures containing G-quartet motifs. These GROs have remarkable growth inhibitory effects against lung cancer cells. We have shown that the antiproliferative activity of GROs is correlated with their binding to a specific cellular protein, nucleolin. Levels of this protein are higher in malignant cells than normal cells, especially lung cancer cells, and nucleolin represents an exciting new target for lung cancer therapy.

We have determined the structure of these new motif GROs using biophysical techniques, molecular modeling, and NMR. We have also identified the specific domains of nucleolin that bind to the GROs, that is the RNA binding domains (RBD) I and 2. We propose to study the structure of RBD12 complexed to GRO26B by NMR, molecular modeling, and biophysical techniques. This will enable us to use structure-based drug design to generate a new class of small molecule agents specific for nucleolin. Small molecules have many advantages over oligonucleotides such as bioavailability, cell entry and stability.

The specific aims are 1) determine the RBD12RGG-GRO26B structure by NMR, 2) use a combination of virtual screening and molecular modeling based on the NMR structure to design small molecules that bind specifically to nucleolin, and 3) test these compounds for inhibition of lung cancer cell growth and competition assays.

Principal Investigator: **Vaclav Vetvicka, Ph.D., University of Louisville**

Research Title: Inhibition of procathepsin D secretion in lung cancer treatment

The long-term objective is to develop a new treatment for lung cancer based on blockade of the autocrine growth factor activity of procathepsin D. Lung cancer cells secrete procathepsin D, the enzymatically inactive form from which the aspartic proteinase cathepsin D is generated by removal of an activation peptide (APpCD). Procathepsin D has been identified as an independent prognostic factor in several forms of cancer, particularly breast, ovarian and prostate cancer. In preliminary experiments, procathepsin D was found to act as a specific autocrine growth factor for lung cancer-derived cells. These effects were mediated through a specific receptor expressed on breast cancer cell lines that is distinct from the usually proposed cathepsin D-specific M-6-P receptor. The region of procathepsin D responsible for its mitogenic activity was localized to amino acids 36-44 of the APpCD sequence.

The proposed specific aims are based on the central hypothesis that procathepsin D is involved in lung cancer via a specific receptor that mediates autocrine activation for increased metastatic growth. For Aim 1 a poorly metastatic human lung cancer cell lines will be transfected with human procathepsin D cDNA, such that the cells will constitutively secrete varying amounts of procathepsin D. The metastatic potential of each transfected cell line will be evaluated both *in vivo* and *in vitro* in relationship to the amount of procathepsin D secreted. In Aim 2 specifically designed ribozymes will be used to inhibit the synthesis and subsequently secretion of procathepsin D in lung cancer cells. After the ribozymes will be tested *in vitro* for cleavage activity, three different human lung cancer cell lines will be transfected with ribozymes and control sequences. Subsequently, the metastatic potential will be evaluated both *in vivo* and *in vitro*.

Principal Investigator: Wolfgang Zacharias, Ph.D., University of Louisville

Research Title: Tumor microenvironment as determinant of protease-mediated malignancy in lung cancer

The focus of this study is to clarify the impact of the balance of cathepsin B and L proteases (CPs) with their natural inhibitors cystatin C and M (CPIs) on lung cancer (LC) tumorigenicity.

Hypothesis: An imbalance between the expression of CPs and CPIs determines invasiveness and metastasis in LC. The intra-tumor microenvironment directly contributes to such observed changes in protease and inhibitor activities in LC.

Specific Aims: The objectives are to establish that CPIs diminish the invasive and metastatic behavior of LC cell lines, and to assess the usefulness of CPIs as therapeutic agents for LC. Special emphasis will be given to analyze factors of the intra-tumor microenvironment on the CP/CPI balance. *Aim 1: To demonstrate that alteration of the CP/CPI balance in LC cell lines will alter their invasive and/or metastatic behavior.* The CP/CPI balance in LC cell lines will be perturbed by expression of inhibitory ribozymes or cystatins, or by overexpression of cathepsins B or L. The resulting consequences on the malignant properties of the modified cells will be analyzed. *Aim 2: To demonstrate that altered growth conditions will effect the CP/CPI balance, and thus the invasive and/or metastatic behavior of LC cells.* The intra-tumor environment will be simulated by hypoxic growth conditions. Alterations of the CP/CPI balance under these conditions will be determined, and the resulting phenotypic changes analyzed by molecular and functional assays. *Aim 3: To establish that cell-cell or cell-matrix interactions contribute to the protease-mediated malignant phenotypes of LC cell lines.* Cell growth at varying cell densities, on different support matrices, or as co-cultures shall simulate the heterogeneous intra-tumor microenvironment. The effects of such tumor-specific factors on the CP/CPI balance, and on the malignant properties of the LC cells, will be determined.

Study Design: The CP/CPI balance will be manipulated by expression of inhibitory ribozymes or cystatins C or M, or by overexpression of cathepsin B or L proteases. The tumor microenvironment will be addressed by analyzing hypoxia, cell interaction, and support matrix effects. As analysis tools, Northern and Western blots, cell proliferation and apoptosis, invasion and enzyme activity assays, and immunohistochemistry will be used.

Principal Investigator: **Stephen G. Zimmer, Ph.D., University of Kentucky**

Research Title: EIF-4E and Metastasis in Lung Cancer

The expression of high levels of the eukaryotic initiation factor eIF-4E is associated with cancer development and has been shown in a number of systems to play a causal role in the biology of cancer cells. Examination of two widely studied lung cancer cell lines indicates that this is true for these cell lines and may indeed be important for their biological behavior. This proposal seeks to study the effect of high 4E levels on the metastatic capacity of these cell lines. Indeed the cell lines will be extensively examined for altered metastatic capacity under conditions of high or low levels of 4E expression using antisense technology. Likewise, the cells will also be examined when the 4E binding protein (4EBP) expression has been restored in these cells. Both approaches result in lowered 4E functional activity that can have a major influence on the expression of mRNAs that are sensitive to 4E levels. Many of these genes are oncogenes, growth regulatory genes, or angiogenesis factors. Thus, many of the mediators of malignancy can be affected at the level of translation. These aspects will be investigated in terms of invasion and metastasis. Once a suitable model system has been developed a search for genes which are altered in their expression in met+ versus met- cells will be done using cDNA arrays. A novel aspect of this search is that the expression of genes sensitive to changes in 4E levels will also be examined using polysome gradients. This approach directly examines mRNAs that exhibit altered translation efficiencies when eIF-4E functional levels are affected. This search will identify both known and unknown genes that could play a specific role in lung cancer metastasis. Finally, a scheme to target selectively tumor cells expressing high levels of 4E is described. The approach is designed to utilize the high expression of 4E in tumor cells as a means of controlling the expression of a tumor suicide gene (the herpesvirus thymidine kinase/Ganciclovir system). The suicide gene would be engineered to be efficiently expressed only in cells expressing high 4E (i.e. the tumor cells). Another approach using a 4E sensitive promoter is also described.