

# Kentucky Lung Cancer Research Program

## Cycle 3 Grant Abstracts

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James L. Wittliff, Ph.D.	UL	Gene Expression Profiling of Human Lung Cancer Cells Isolated by Laser Capture Microdissection

Principal Investigator: **Susanne M. Arnold, M.D., University of Kentucky**

Research Title: Low-dose fractionated radiation plus Docetaxel and Cisplatin as induction therapy for stage II and IIIA non-small cell lung cancer

Surgical treatment for non-small cell lung cancer (NSCLC) has limited success in curing stage II and IIIA disease, with 5-year survival rates of 24% and 10%, respectively. While neoadjuvant chemotherapy has increased the 5-year survival rate of stage IIIA patients to 30%, emerging data indicates that the neoadjuvant approach may provide even greater benefit in disease-free and overall survival in early stage NSCLC. Synergy between radiation and chemotherapy is well established in NSCLC. Because it has demonstrated improved survival in locally advanced lung cancer, the combination of neoadjuvant chemotherapy and radiation would be expected to provide even greater local control and survival benefit in earlier stage disease, but has always been thought to be too toxic. At the cellular level, low doses of radiation (< 50 Gy) impart effective cell killing, termed hyper-radiosensitivity (HRS). In this setting, low dose radiation exerts a unique biologic effect—it causes apoptosis without inducing pro-survival pathways, a common cause of resistance to cancer therapies. Original preclinical work from the University of Kentucky reveals that fractionating the low doses of radiation in various carcinoma cell lines enhanced apoptosis especially when combined with chemotherapy. Translating this observation into the clinical setting, a novel strategy of chemotherapy and low dose fractionated radiation (LDFRT) has been designed as neoadjuvant therapy in stage II and IIIA NSCLC. The central hypothesis of this proposal is that combining chemotherapy with the lowest possible dose of radiation will augment response and enhance survival in early stage lung cancer without increasing toxicity. In addition, investigation of the mechanisms of this chemopotential is proposed, specifically, with respect to p53- and TGF- $\beta$  mediated pathways and cross-point regulators of apoptosis.

Principal Investigators: **Howard P. Glauert, Ph.D., Brett T. Spear, Ph.D.,  
University of Kentucky**

Research Title: Antioxidants, NF- $\kappa$ B, and Cigarette Smoke

Dietary antioxidants have been shown to inhibit carcinogenesis in many studies, but the mechanisms by which they do so are unclear. One possibility is that they influence the activation of oxidative stress-sensitive transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B). Our preliminary studies have shown that cigarette smoke constituents are capable of activating NF- $\kappa$ B in a variety of cultured cells, including lung epithelial cells. In this project, we propose to test the hypothesis that antioxidants inhibit smoke-mediated *in vitro* and *in vivo* NF- $\kappa$ B activation in the lung, and that NF- $\kappa$ B activation is necessary for lung cancer induction by cigarette smoke. We propose to study antioxidants such as vitamin E,  $\beta$ -carotene, lycopene, coenzyme-Q10, N-acetyl cysteine, and curcumin. The ability of antioxidants to inhibit cigarette smoke condensate (CSC)-induced DNA binding activity of NF- $\kappa$ B and activity of NF- $\kappa$ B-regulated reporter genes will be first tested *in vitro*. Those antioxidants that are found to inhibit CSC-induced NF- $\kappa$ B activation *in vitro* will be tested for their effectiveness *in vivo*. NF- $\kappa$ B activation, lung epithelial cell proliferation, and lung epithelial cell apoptosis will be examined in control and smoke-exposed mice with the absence and presence of dietary antioxidants. Finally, an essential role of NF- $\kappa$ B activation in cigarette smoke-induced lung tumorigenesis will be determined using a mouse deficient in the p50 subunit of NF- $\kappa$ B. These studies will demonstrate if NF- $\kappa$ B is important in the development of lung cancer and if its activation can be inhibited by dietary antioxidants. These results may provide a mechanistic basis for possible dietary recommendations for the prevention of lung cancer.

Principal Investigators: **Sham S. Kakar, Ph.D., University of Louisville**

Research Title: Molecular Mechanisms of PTTG in Lung Cancer

Lung cancer is the leading cause of cancer related death for both men and women in the United States and the solid tumor with the most defined relationship to a known environmental cause, cigarette smoking. Kentuckians have the highest incidence of both smoking and deaths due to lung cancer. Although this disease is treatable if detected at an early stage, it is often not diagnosed until the disease has advanced.

Given the poorly defined hereditary aspects of lung cancer, little is known about the precise gene alternations that underlay the earliest steps in lung tumorigenesis. Accordingly, there is a pressing need to understand the development of lung tumors at the molecular level.

We reported recently on the isolation of a novel oncogene (PTTG; pituitary tumor transforming gene) from human testis. This gene is highly over-expressed in all lung tumors and cell lines derived from these tumors that have been analyzed to date, whereas expression of PTTG in normal lung tissues is either low or undetectable. Our initial research and the research of other scientists suggest a potent oncogenic function for PTTG.

Our efforts with this research project focus on understanding the molecular role of PTTG in lung tumorigenesis and its mechanisms in tumor progression and metastasis. The results we obtain will provide critical information that will be used for the development of new strategies of treatment for lung cancer.

Principal Investigator: **Kyung-Bo Kim, Ph.D., University of Kentucky**

Research Title: Molecular Mechanism of an Apoptosis-Inducing Estrogen Metabolite 2-Methoxyestradiol in Lung Cancer Cells

Biologically active natural products continue to be useful in the exploration and control of intracellular signaling processes. Moreover, the mode-of-action studies of bioactive natural products may lead to the discovery of novel targets for therapeutic intervention. In order to identify novel targets for drug design and to investigate regulatory mechanisms in lung cancer, we have chosen 2-methoxyestradiol, which induces apoptosis of human lung cancer cells in a p53-dependent and estrogen receptor-independent pathway but not human normal bronchial epithelial cells.

Specifically, the objectives of this project proposed here are 1) to synthesize an affinity reagent-tagged 2-methoxyestradiol; 2), using this reagent, to identify the 2-methoxyestradiol-binding protein(s) in human lung cancer cells and 3) to clone the identified target protein(s) with which signaling pathways involved in the proliferation of human lung cancer cells will be characterized using various biochemical and cell biological tools.

The results of these studies proposed here will provide a detailed description of 2-methoxyestradiol intracellular target(s) and, ultimately, their molecular mechanisms of action, enhancing our understanding of the regulation of lung cancer cell proliferation and how they can be controlled pharmacologically.

**Principal Investigators: Kenneth L. Kirsh, Ph.D., Steven D. Passik, Ph.D.,  
University of Kentucky**

**Research Title:** Testing a Strategy for Early Intervention and Prevention of Depression in Lung Cancer Patients: Impact on Multiple Symptoms and Quality of Life

Cancer patients typically have multiple symptoms that negatively effect quality of life, and depressive symptoms are often under-recognized and undertreated. With the average patient having 10 symptoms, it is important to find treatments with economy of function for symptom management. To this end, we hope to undertake a novel and potentially groundbreaking study to explore the effects of mirtazapine (Remeron ®) as a prophylactic treatment for preventing depression, by employing it early as a treatment for insomnia. We have previously shown that mirtazapine has potential benefits for appetite, weight maintenance and gain, energy, daytime sleepiness, pain, and overall quality of life. We chose to screen for insomnia since it is common in this population (40-60%), which offers us a chance to treat a large number of people, alleviate the symptom of insomnia, and potentially help with other symptoms and preempt the development of depression. Interventions for insomnia have the potential to dramatically improve quality of life, although this issue has been poorly studied. To date, no prophylactic study of antidepressants for the prevention of major depression has ever been attempted in lung cancer patients.

Principal Investigator: **David K. Orren, Ph.D., University of Kentucky**

Research Title: Responses of replication, recombination, and checkpoint signaling pathways to unrepaired PAH adducts in DNA

Polycyclic aromatic hydrocarbon (PAH) components of tobacco smoke are strongly linked to lung cancer in humans. After activation by cellular metabolism, PAHs react with DNA to generate bulky adducts that interfere with replication and, under some circumstances, cause mutations. Thus, PAH exposure increases the burden of DNA damage, the accumulation of mutations with age, and the probability that mutations will alter cellular proliferation and result in cancer. To date, research has focused on metabolic activation of PAHs, generation and stereochemistry of PAH adducts, and induction of mutations after PAH treatments. However, the steps that fall between persistence of DNA adducts and eventual fixation of mutations remain unclear. Experiments with model DNA substrates indicate that PAH adducts are strong blocks to purified DNA polymerases and infer that PAH adducts in the template block cellular replication. Moreover, damaging agents other than PAHs appear to block replication fork movement in cells. Theoretically, replication blockage can be resolved by recombination or translesion synthesis pathways. In addition, replication blocks may activate checkpoint signaling pathways to delay cell cycle progression or induce apoptosis. Recent studies show recruitment of recombination and checkpoint signaling factors to sites of blocked replication, suggesting involvement of these pathways in their resolution. However, this has not been investigated using PAH treatments, and mutations generated by PAHs are now thought to occur via translesion synthesis by specialized bypass polymerases. This proposal examines the relationships between persistent PAH adducts (generated by benzo[a]pyrene and dibenzo[a,h]pyrene metabolites), replication blockage within cells, and subsequent cellular responses such as recombination, checkpoint signaling, and translesion synthesis. Cell lines deficient and proficient for repair of PAH adducts will be investigated to correlate DNA adduct levels to replication blockage and subsequent events, then compared to define the effects of adduct repair. The channeling of replication blockage by PAH adducts into downstream pathways will be investigated by determining the timing and recruitment of proteins factors to sites of blocked replication. These approaches will examine the repair or persistence of various PAH adducts, determine the relationship between persistent PAH adducts and replication, and take initial steps to define how adducts and blocked replication forks may be channeled into mutation avoidance or induction pathways. Once these events become known, strategies to lower mutational load and thus cancer incidence can be tested.

**Principal Investigator: Mariusz Z. Ratajczak, M.D., Ph.D., D.Sc., University of Louisville**

**Research Title: The Role of Microparticles in Lung Cancer Progression, Angiogenesis and Metastasis**

This project is driven by a new original hypothesis that microparticles or microvesicles (MV) derived from tumor cells and platelets play an important under-appreciated role in lung cancer progression, metastasis, and vascularization. The presence of circular membrane fragments (microvesicles) shed from the cell surface or secreted by the tumor cells and activated platelets in tumor tissue was observed for many years but no attention was paid to the potential biological significance of this phenomenon.

Similarly, we knew that platelets may increase the metastatic potential of tumor cells; however, no attention was paid to the role of platelet-derived microvesicles in this process. In addition, we recently identified a new potent role which MC may play in tumor angiogenesis. We believe by clarifying microvesicle-related mechanisms in cancer progression, metastasis and angiogenesis we will open a new area of investigation of lung cancer.

We will isolate MV shed from the cell surface or secreted as exosomes by the tumor cells and platelets. We will evaluate the influence of these MV *in vitro* on proliferation, survival, adhesion, secretion of angiopoietic factors by lung cancer and endothelial cells. Our *in vivo* experiments in a mouse model will focus on the role of MV in metastasis and angiogenesis.

Principal Investigator: **Mitzi Schumaker, Ph.D., University of Kentucky**

Research Title: The Comprehensive Support Protocol: Providing Psychosocial Assistance to Lung Cancer Patients and Their Families

Health care research has long emphasized the psychosocial ramifications of chronic illness for patients and family members. However, few studies have developed programs designed to provide comprehensive psychosocial support to these individuals. The objective of the proposed study is to implement and evaluate the Comprehensive Support Protocol (CSP) over a 3-year period for individuals with lung cancer as well as their family members. The CSP includes the following treatment components: 1) 6 sessions of individual/family counseling; 2) ongoing support groups; and 3) ongoing ad hoc counseling, where family members or patients can meet with or call a counselor at any time. A sample of 100 dyads that include people with lung cancer and their 'primary' family members (i.e., the family member who provides the most emotional and instrumental assistance to the person with cancer) will be recruited from the University of Kentucky Markey Cancer Center to participate. Fifty dyads will be randomly assigned to the CSP treatment condition and 50 dyads will be assigned to a control group where usual clinic services are offered. A research assistant will administer interviews prior to randomization, 4 months, 8 months, 12 months, and every 6 months thereafter to collect information. The longitudinal data available will allow for state of the art analytical techniques (e.g., growth curve modeling) that evaluate the influence of the CSP on outcomes of interest (e.g., stress, depression, quality of life). The proposed program will offer a potentially effective service to families who must cope with and manage lung cancer. This innovative approach to support will also provide a portable service that professionals can adapt and implement to help families in need across health care contexts.

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

Research Title: Functional Polarization of Tumor Infiltrating Macrophages

One of the major mechanisms by which malignant cancer cells evade destruction by the immune system is to produce or cause the production of molecules that inhibit or prevent immune responses. Macrophages are one of the cell types of the immune system that participate in all stages of the immune response. When a tissue is injured, macrophages arrive very early and begin to intensify inflammation to increase the number of immune cells entering the injured tissue.

Macrophages promote immune responses to destroy any invading bacteria. Once the bacteria have been destroyed, macrophages display the opposite function; they inhibit inflammation and mobilization of the immune system (now no longer needed) and promote tissue regeneration—wound healing.

Our research examines the hypotheses (1) that malignant tumors stimulate macrophages to display the second type of activity—to inhibit inflammation and promote tissue growth; and (2) that the effect of the tumor on macrophages is reversible and therefore may be countered by delivering molecules that strongly stimulate inflammation and mobilization of immune responses.

Overall, this research will increase our understanding of how tumors change macrophage inflammatory functions and thereby promote tumor rejection.

Principal Investigator: **Hsin-Hsiung Tai, Ph.D., University of Kentucky**

Research Title: Prostaglandin Dehydrogenase and Lung Cancer

Available evidences indicate that aberrant expression of the enzymes involved in arachidonic acid metabolism may contribute significantly to the development of lung cancer. Notable examples are the increased expression of cyclooxygenase-2 (COX-2) and PGE<sub>2</sub> synthase in non-small cell lung cancer (NSCLC) resulting in elevated level of PGE<sub>2</sub> which may induce local immunosuppression, a condition that favors tumor growth. However, level of PGE<sub>2</sub> is not only controlled by synthetic enzymes but also by catabolic enzymes, a fact that has been overlooked in studying prostaglandins and cancer. We, therefore, hypothesize that NAD<sup>+</sup>-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the key enzyme involved in biological inactivation of PGE<sub>2</sub>, is down regulated in NSCLC resulting in an impaired catabolism of PGE<sub>2</sub>. Accordingly, 15-PGDH may be considered an anti-oncogene. We plan to provide evidences that 15-PGDH may act as an anti-oncogene both in human lung adenocarcinoma A549 cells as well as in a murine cancer model. Specific aims include: (1) Is 15-PGDH expression down regulated in human lung cancer? (2) Generation and characterization of 15-PGDH overexpressing A549 cells; (3) Does overexpression of 15-PGDH in A549 cells lead to inhibition of tumorigenesis in athymic mice? The results of the proposed research should provide convincing evidences that 15-PGDH may act as an anti-oncogene and that 15-PGDH has the potential to become a novel therapeutic for clinical cancer gene therapy.

Principal Investigator: **James L. Wittliff, Ph.D., University of Louisville**

Research Title: Gene Expression Profiling of Human Lung Cancer Cells Isolated by Laser Capture Microdissection

Lung carcinoma is the most common cause of cancer deaths in the United States and other developed countries. More than 186,000 cases of lung carcinoma will be reported in year 2002, with approximately 80% presenting as non-small cell lung cancer and the remaining 20% diagnosed as small cell lung cancer. It is important in terms of clinical management of patients to distinguish between the various subtypes.

The goal of this study relates to the fact that for routine pathology examination of cancer biopsies, formalin-fixation, and subsequent paraffin-embedding are used to process the tissue. Furthermore, the number of tissue biopsies processed annually is enormous; for lung cancer alone over 1 million biopsies are performed each year in the United States.

Tissue specimens preserved in paraffin blocks are extremely stable, permitting subsequent DNA and certain protein (limited to immunohistochemistry) analyses for many years after surgical removal. In contrast, formalin-fixed specimens have been poor materials for subsequent mRNA (nucleic acid) studies.

There is a worldwide need to establish a procedure for extracting high quality, high yield mRNA from formalin-fixed, paraffin-embedded tissues for use in gene chip (DNA microarray) assays which profile the activities of thousands of genes simultaneously. This advanced assay gives a "snap shot" of the cellular physiology of a specimen, which we propose will be helpful in clinical management of lung cancer.

Currently only frozen tissue samples are being used for microarray analyses of lung biopsies. Our study is designed to solve the use of formalin-fixed, paraffin-embedded biopsies and DNA microarrays, with the long-term goal of assisting other investigators in accessing huge repositories of formalin-fixed clinical samples to determine the potential of microarray analyses of routine samples of the diagnosis, assessment of prognosis (clinical course of the disease) and therapy selection, thereby improving clinical management of patients with lung cancer.