Research Interest:

Environmental Pollutants and Lung Diseases

Our current research focuses on the regulation of lung surfactant biosynthesis and secretion. This involves studies on the purification and molecular cloning of cholinephosphotransferase, the terminal membrane-bound enzyme for the biosynthesis of phosphatidylcholine, a main component of lung surfactant. We are also interested in how lung surfactant secretion is regulated by adrenergic and peripheral benzodiazepine receptors and annexins. Studies are ongoing to develop animal model on lung diseases, such as COPD, Emphysema and ARDS. We are particularly interested in elucidating the molecular mechanisms by which exposure of mainstream and sidestream cigarette smoke and mustard gas, a chemical warfare agent, causes airway hypersensitivity and lung diseases. To achieve this goal, we are studying several cell signaling pathways using biochemical, molecular biology and immunological techniques. Our long term goal is to develop innovative therapeutic approaches to prevent lung diseases caused by vasculotoxic agents.

Soy and Breast Cancer: Food for Thought

Dietary factors may be among the environmental aspects influencing the development and progression of breast cancer. It has been established that women in Asian countries consume more soybean products than women in the United States and that the incidence of breast cancer in women in Asian countries is generally lower. While this association is correlative and no causative effect has been demonstrated, an increasing body of evidence suggests that soybean products consumption may be protective, thus reducing the risk of breast cancer development. A major research focus in my laboratory during the last twelve months has been on the development of a DMBA-induced rat breast cancer model and elucidation of the molecular mechanism by which soybean protein exerts it breast cancer protective effect. The protective effect of soy is associated with a decrease in the expression of a cancer promoting genes, peripheral benzodiazepine receptors (PBRs) and a PBR-dependent decrease in angiogenic signaling molecules. PBR expression is increased in human breast cancers and is associated with an aggressive course. Thus, targeting PBR may have clinical importance.

Endothelin-1 as Potential Biomarker of Breast Cancer Aggressiveness

Triple negative breast cancer (TNBC) is an aggressive tumor phenotype with higher prevalence in African American (AA) women as compared to Caucasians (C) women (1). AA women with TNBC have worse clinical outcomes with higher frequency of metastasis to the lung, liver and brain, and high mortality rate (2). Elevated expression of endothelin-1 (ET-1) and its receptors ETAR and ETBR have been detected in a variety of malignancies where they promote tumor growth, progression and metastasis (3-5). Our preliminary data demonstrated that expression of ET-1, ETAR and ETBR genes is elevated in TNBC tumors. Analysis of cell lines and primary breast cancer (BC) tissue showed that ET-1 is strongly expressed by tumor cells, while its receptors ETAR and ETBR are present on both tumor cells and tumor vasculature. These data suggest that ET-1 signaling can modulate both tumor cells and the tumor microenvironment. Furthermore, TCGA analysis indicates that increased signaling of ET-1, ETAR, ETBR as well as ECE-1 (endothelin converting enzyme 1) is associated with the increased expression of pro-inflammatory genes. Of note, inflammation has been shown to facilitate BC progression and metastasis (6). Even though it is well established that ET-1 and its receptors ETAR and ETBR play an important role in breast cancer aggressiveness, it is not known whether their expression differs between the subtypes of TNBC. A limited information is available on whether downregulation of ET-1 expression by treatment with ET-1 receptor antagonist and/or silencing of ET-1 by shRNA in TNBC patients will reduce the tumor burden and its metastatic spread. **The objective of this study is to test the hypothesis that (1) the expression of ET-1 and its receptors is different among subtypes of TNBC, (2A) ET-1 and its receptors play a direct role in proliferation and aggressiveness of TNBC cells, and (2B) ET-1 plays a role in establishment of tumor-promoting microenvironment**. To accomplish Aim 1, we will study RNA expression bioinformatically – taking the TCGA TNBC samples, dividing them into subtypes per the Pietenpol criteria, and then assessing the mRNA expression levels of ET1 axis members from the TCGA data. To accomplish Aim 2A, we will obtain 12 TNBC cell lines from ATCC (5 of AA and 7 of C) and compare with non-transformed cell lines, MCF-10A, MCF-12A and MCF-12F, all of C origin as control. We will carry out various functional assays by treating the cells with ETR antagonists or by transfection with ETAR/ETBR siRNA under similar conditions. If silencing the ET receptors in TNBC cell lines causes a decrease in the expression

and protein level of ET-1 and its receptors with simultaneous modification of their functionality, it will offer a mechanistic link. For determination of the expression of ET-1 axis, we will use Real-Time PCR, immunohistochemistry, Western Blot and FACS analysis. For studying functionality of ET-1 and its receptors in terms of tumor proliferation and aggressiveness (Aim 2A), we will conduct cell proliferation, cell cycle progression, and immunofluorescence studies for Ki-67, cyclin D, VEGF, and E-cadherin as well as by monitoring of the AKT, MAPK/ERK, PKC and EFGR signaling that regulate the epithelial mesenchymal transition process, which is the early event of cancer cell invasion. Furthermore, to establish the direct role of ET-1 in establishment of tumor-promoting microenvironment (Aim 2B), we will knockdown the ET1 gene in EO771 cells and 4T1 cells (both TNBC) using shRNA lentiviral vectors and inject the cells into mammary fat pads of C57B1/6 and BALB/c mice. Resulting ET-1 deficient and ET-1 expressing tumors will be compared in respect to size, weight, and vascularization. Tumor-bearing animals will undergo full necropsy and the number and size of metastatic lesions in lungs, liver, bones and tumor-draining lymph nodes will be evaluated by a pathologist. Tumor immune infiltrate will be analyzed by flow cytometry and IHC staining detecting immune cell markers. Expression of pro-inflammatory molecules will be studied using mRNA profiling and cytokine arrays. Targeting such molecular factor might be an attractive approach in predicting and controlling the aggressiveness of TNBC among AA population.