AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2016 – 2018

TITLE: Phytochemical Supplement form Cruciferous Vegetables and Protection of the Fetus form Exposure to Carcinogens: Role of Long Non-Coding RNAs

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COOPERATORS: NA

EXECUTIVE SUMMARY: Mice exposed *in utero* to the polycyclic aromatic hydrocarbon (PAH), dibenzo[*def,p*]chrysene (DBC, a probable human carcinogen) develop lung cancer upon reaching 10 months of age. Sulforaphane (SFN), a phytochemical from cruciferous vegetables is chemopreventive against PAH-dependent lung cancer. Our transplacental chemoprevention model involves treating pregnant mice with DBC and supplementing the maternal diet with SFN which has recently been shown to alter the expression of long non-coding (lnc)RNAs associated with cancer. We found a number of lncRNAs were altered by SFN in a gender specific manner in tumor compared to normal tissue from adult mice exposed to DBC *in utero*.

OBJECTIVES: Using a commercially available qtPCR array, examine the profile of lncRNAs in tumor tissue and normal lung from lung of offspring exposure *in utero* to DBC from dams fed control diet or SFN. Determine if SFN-dependent alterations were a function of Nrf2 expression (mice were *Nrf2*$^{+/-}$ or *Nrf2*$^{-/-}$)

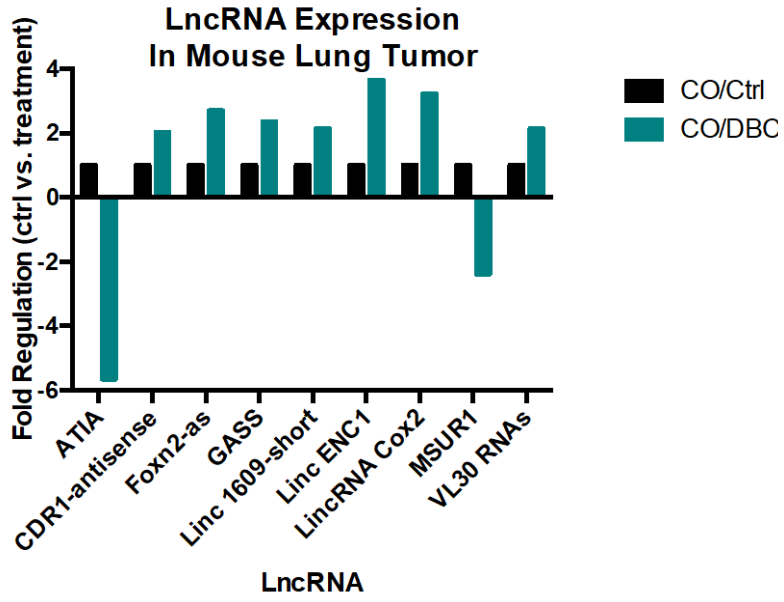

Figure 1. Alteration in Expression of lncRNAs Associated with Cancer in Normal vs Lung Tissue from Mice Treated with DBC *In Utero*

PROCEDURES: The System Biosciences, Inc. (SBI) Mouse lncRNA array (RA930A-1), was utilized for these studies to assess the impact of fetal exposure to DBC when the mother was fed SFN on lncRNA expression in normal and tumor tissue at 10 months of age of mice genotyped as *Nrf2*$^{+/-}$ or *Nrf2*$^{-/-}$. This array measures expression of 90 lncRNAs known to be important in cancer. The normal lung tissue and tumor

samples (from the same mouse) were archived from a previous study. Total RNA was isolated, quantified using an ND-1000 spectrophotometer and the quality determine with an Agilent 2100 bioanalyzer. The amount of each lncRNA in each treatment group is then determined by quantitative PCR (qPCR) using SYBR green dye.

SIGNIFICANT ACCOMPLISHMENTS: We demonstrated, for the first time, that if fetal mice were exposed to a transplacental carcinogen (DBC) a marked difference in expression of lncRNAs, known to be associated with lung cancer, could be detected in mouse lung tumors relative to adjacent normal tissue (Figure 1). If the pregnant dam was fed SFN, 10 month old offspring demonstrated down-regulation of a number of lncRNAs (e.g., H19 antisense, Foxn2-as, Gtl2-as, Linc ENC1, LincRNA Cox2) that were altered in lung tumor tissue from mice exposed to DBC *in utero*. There were some gender-specific effects which is not surprising given the difference in susceptibility to lung cancer in male and female mice. Surprisingly, the attenuation of cancer-related lncRNAs by SFN was not dependent upon the *Nrf2* genotype.

BENEFITS & IMPACT: The Developmental Origins of Health and Disease hypothesis has shown that exposure to chemicals and other stressors in the fetus and infant play an important role in susceptibility to adult disease. Modulation of the maternal diet with chemopreventive agents, such as cruciferous vegetables (an important crop in Oregon) or the phytochemical components found in those foods, provide protection for the fetus/infant from chemical carcinogens that can cross the placenta and/or be transferred through breast milk. As the developing fetus is very susceptible to epigenetic mechanisms of gene expression, it is not surprising that exposure to these chemical carcinogens can alter the expression of lncRNAs thought to be important in development of cancer. We have shown that supplementation of the maternal diet with SFN from cruciferous vegetables can attenuate the impact of chemical carcinogens on lncRNA expression. A long-term goal would be to develop chemoprevention strategies to protect the fetus and infant with supplementation of the maternal diet with functional and beneficial foods. The expression of lncRNAs could be used as early biomarkers of cancer susceptibility and its modulation by diet.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES: The PI, along with Drs. Emily Ho and Richard van Breeman (new Director of the Linus Pauling Institute) are planning a submission to the National Cancer Institute to renew a program project grant ("Comparative Mechanisms of Cancer Chemoprevention) which utilized this mouse transplacental chemoprevention model and generated the archived lung tissue samples used in this study. We plan to focus on epigenetic mechanisms of cruciferous vegetable modulation of gene expression early in development including microRNAs, DNA methylation and lncRNAs.