AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

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

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SUMMARY: Exposure to toxic environmental chemicals early in life (the "Early Life Stage Exposures and Adult Disease" hypothesis) enhances susceptibility to cancer later in life. The fetus and infant are especially sensitive to *in utero* and lactation exposures. A dietary supplement to protect the fetus/infant from chemical carcinogens would benefit human health and be "value added" for Oregon crops (Brussels sprouts, broccoli, kale, cauliflower, cabbage, horseradish, etc.). The focus is on epigenetic mechanisms of protection which can be passed through multiple generations; a pregnant woman consuming cruciferous vegetables or a supplement derived from these plants could provide her baby with protection and also her grandchild and great-grandchild. Feeding a pregnant mouse indole-3-carbinol (I3C), a commercial dietary phytochemical supplement from cruciferous vegetables, provides significant protection for offspring against cancer from *in utero* exposures. These cancers include leukemia, the number 1 cause of cancer deaths in children. One hundred percent of the offspring also have multiple lung cancers (the number 1 cause of cancer deaths in the U.S., also with a poor (15%) survival rate) at 10 months of age (equivalent to middle age in human). Additional cancers caused by *in utero* exposure include liver and ovarian. I3C in the maternal diet provides protection against these cancers. Even though the offspring are never exposed; only the mother. The purpose of this study is to how maternal dietary I3C could alter gene expression in the fetus/infant with a focus on the epigenome, specifically long non-coding RNAs (lncRNAs). LncRNAs used to be considered "junk" DNA but now are accepted to be important in development and cancer.

OBJECTIVES: Objectives: The objectives of the proposed study are:

(1). Using a commercially available array, examine the profile of lncRNAs in lung from 1-day old mice born to pregnant mice exposed to a carcinogenic polycyclic aromatic hydrocarbon (PAH) or mice treated with the PAH but fed I3C in their diet.
(2). Perform an identical evaluation on offspring that survive to 10 months of age and develop lung tumors. Determine the difference in expression between normal lung tissue and lung tumors in the same mice.
(3). Compare the profiles in (1) and (2) to determine if I3C-dependent changes are maintained through "middle age", an indicator that these protective epigenetic mechanisms can be passed to subsequent generations.

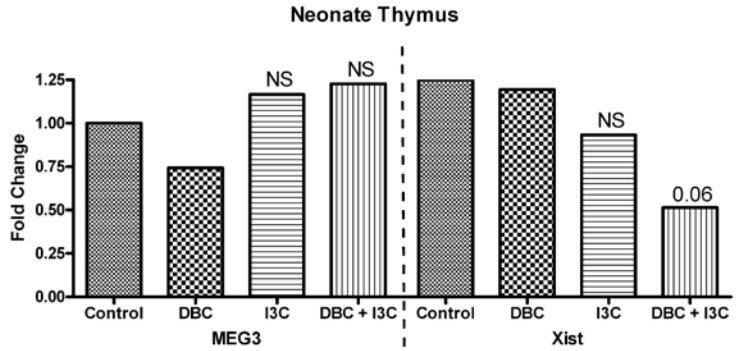

Figure 1. MEG3 and Xist lncRNA in Newborn Thymus

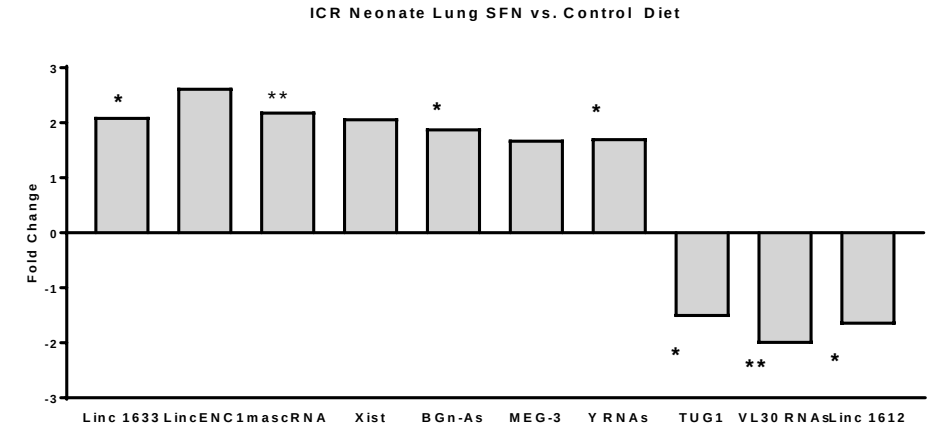

Figure 2. lncRNA Levels in Lungs from Newborns from Mothers Fed SFN Compared to Control Diet

PROCEDURES: Archived tissues from two previously-conducted mouse transplacental studies were analyzed for changes ncRNAs. In both studies pregnant mice were a Class 2A (probable human carcinogen, dibenzo[*def,p*]chrysene (DBC)) while fed a diet containing I3C and/or sulforaphane (SFN, also from cruciferous vegetables). A subsample of newborn thymus, lung, and liver was harvested immediately after birth; remaining progeny monitored for 10 months and evaluated for leukemia and cancer of the lung, liver and

Table 1. lncRNAs Impacted by Maternal Dietary Sulforaphane http://www.lncrnadb.org/mascRNA/

Name	**Functions and Associated Elements**
LincENC1	Regulates embryonic stem cell cycle and viability.
mascRNA	Small cytoplasmic RNA associated with Malat-1 (a predictor of metastasis in lung cancer).
MEG3	Putative tumor suppressor, activates p53 and inhibits cell proliferation
TUG1	Contains p53 binding site in its promoter, DNA damage activates via p53.
VL30 RNAs	Binds to PSF protein which together regulate steroidogenesis, proto-oncogene transcription, cell proliferation, and tumorigenesis.
Xist	Regulates balance of X-linked gene expression.

ovary. Tissues were collected from surviving animals to 10 months of age. During the first year of this project lncRNA levels have been evaluated in neonate thymus and lung from study #1 and neonate lung from study #2 (different mouse strains). We used a lncRNA Mouse Cancer Profiler qPCR array from System Biosciences, Inc. (Palo Alto, CA.) to measure levels of selected lncRNAs thought to be associated with cancer.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: Figure 1 shows the fold change in MEG3 and Xist lncRNA in neonate thymus (leukemia). DBC decreased MEG3 whereas I3C caused an increase. Xist also decreased with the addition of I3C to maternal diet. At this time lung tissue is still being evaluated from study #1. We also compared lung tissue of newborn mice from mothers fed SFN (Figure 2). Addition of SFN to the diet up-regulated Linc 1633, Linc ENC1, mascRNA, Xist, BGn-As, MEG3, and Y RNAs. An additional three, TUG1, VL30 lncRNAs, and Linc 1612 were down regulated (* indicates $p \leq 0.05$). Table 1 lists a few known functions and associated proteins for the selected lncRNAs. We also looked at select microRNAs (miRNAs) in liver tumors and control liver from 10 month-old mice. miRNAs are small (18-22bp) ncRNAs that regulate gene expression post-transcriptionally and play critical roles in development and progression of liver cancer. miRNA have three primary functions in cancer; promotion or inhibition of growth, metastasis, and apoptosis. Based on the literature we chose mir-181c and mir-221. We used a miScript Primer Assay (Qiagen) to compare DBC treated livers from both control and SFN diets. Both miRNAs were up-regulated in tumors compared to control tissue (not shown). Addition of SFN in the diet did not change expression levels. In study 2 there was significant liver tumor incidence at 10 months in male, but not female offspring. It is difficult to determine sex in neonates harvested immediately at birth. To evaluate early changes in ncRNAs in neonates we are developing methods for determining sex with PCR and plan to look at male neonate livers.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: None

FUTURE FUNDING POSSIBILITIES:

Pending NIH grant, “Genotoxicity and Epigenetics in PAH Transplacental Cancer: Chemoprevention by I3C”, (R01 ES028471-01), 07/01/2017-06/30/2022, $2,685,789, total costs; to be reviewed in the February/March meeting of special study section (ZRG1 DKUS-P (02).