**TITLE:** Epigenomic Adaptation to Low Dose Radiation

**DOE NUMBER:** DE-SC0010028 4/1/13-3/31/14 with No Cost Extension awarded 4/1/14-3/31/15

**FUNDING AMOUNT:** $263,390.00

This grant was submitted in order to complete the project originally begun by Dr. Randy Jirtle at Duke University. Dr. Jirtle continued to work on this project through his appointment as a Visiting Professor/Scientist at the University of Wisconsin-Madison, Department of Oncology. Dr. Michael Gould, from this same department, headed the completion of the project as PI. Together Drs. Gould and Jirtle worked with Jill Haag at the University of WI-Madison to complete Specific Aim 3 of this project during the final year of funding.

**PROJECT NARRATIVE:**

The overall hypothesis of this grant application is that the adaptive responses elicited by low dose ionizing radiation (LDIR) result in part from heritable DNA methylation changes in the epigenome. In the final budget period at the University of Wisconsin-Madison, we will specifically address this hypothesis by determining if the epigenetically labile, differentially methylated regions (DMRs) that regulate parental-specific expression of imprinted genes are deregulated in agouti mice by low dose radiation exposure during gestation. This information is particularly important to ascertain given the 1) increased human exposure to medical sources of radiation; 2) increased number of people predicted to live and work in space; and 3) enhanced citizen concern about radiation exposure from nuclear power plant accidents and terrorist 'dirty bombs.'

**PERSONNEL**

| | | |
|---|---|---|
| Michael N. Gould | PI | 10% salary/fb |
| Randy L. Jirtle | Scientist | 25% salary/fb |
| Jill D. Haag | Researcher | 50% salary/fb |

**DOE SUMMARY**

- We transferred Dr. Jirtle's viable yellow agouti ($A^{vy}$/a) mice to UW-Madison, and established a breeding colony, along with rederiving the $A^{vy}$/a mice, and establishing an independent breeding colony in a Level 1 specific pathogen free facility at WIMR.
- We tested and improved the PCR based genetic screening for the agouti mice that allowed us to create and derive a line of $A^{vy}/A^{vy}$ homozygous mice.
- The $A^{vy}/A^{vy}$ homozygous male mice were used to generate $A^{vy}$/a offspring more efficiently in the studies designed to reproduce some of the experimental data that Dr. Jirtle previously published in 2013 (1).
- We used the following protocol at UW-Madison to reproduce data published by Dr. Jirtle.
    - $A^{vy}/A^{vy}$ homozygous males X a/a (C57BL/6J) female mice consuming Teklad TD.95092 diet were bred overnight. Teklad TD.95092 replaces soybean oil with 7% corn oil to reduce effects of isoflavones (i.e. genistein) on the $A^{vy}$/a phenotypes (2).
    - Detection of vaginal plugs the next morning was determined to be day 0.5.
    - Pregnant females were irradiated with 3.0 cGy intra-uterine dose at day 4.5 gestation in a XRAD irradiator at the WIMR facility or sham-irradiated.
    - Approximately 1 week after irradiation, the pregnant female mice were transported to the MSC facility where the Gould lab was located.

- Dates of birth and number of pups per litter were recorded.
- At 23 days of age (+/- 2 days), each offspring ($A^{vy}$/a) was euthanized, sexed, scored for coat color, and liver tissue were collected and frozen in liquid nitrogen for future DNA analysis.
- Coat color classifications used:
  - CC5 = pseudoagouti or >95% brown
  - CC4 = heavily mottled or 60-95% brown
  - CC3 = mottled or ~50% brown
  - CC2 = slightly mottled or 5-40% brown
  - CC1 = yellow or <5% brown

❖ We confirmed the following effects of *in utero* low dose ionizing radiation exposure on the coat color of $A^{vy}$/a mice.

- There was a doubling in the percentage of pseudoagouti mice (CC5 – Brown) when $A^{vy}$/a mice were exposed *in utero* to 3.0 cGy of low LET radiation (Table 1). This finding is consistent with that previously reported (1).

**Table 1:** Effect of LDIR on the coat color of $A^{vy}$/a offspring.

| | Sham Irradiation | 3.0 cGy |
|---|---|---|
| All Mice CC5 - Brown | n=7/52 13% | n=8/31 26% |
| Males CC5 - Brown | n=4/25 16% | n=4/12 33% |
| Females CC5 - Brown | n=3/27 11% | n=4/19 21% |

- There was also evidence that the effect of radiation on the incidence of pseudoagouti mice (CC5 – Brown) is more pronounced in male than in female offspring (Table 1), as previously demonstrated (1).

❖ We demonstrated that a maintenance diet containing soybean oil (Teklad 8604 Diet) increased the incidence of pseudoagouti offspring (CC5 – Brown) when compared to that obtained when the mothers ate a diet where the soybean oil was replaced with corn oil (Teklad TD.95092 Diet) (Table 2). This is consistent with the finding that maternal dietary genistein supplementation of mice during gestation shifts the coat color of $A^{vy}$/a offspring toward pseudoagouti by modifying the fetal epigenome (2).

**Table 2:** Effect of maternal diet on the coat color of $A^{vy}$/a offspring.

| | Teklad 8604 Diet | Teklad TD.95092 Diet |
|---|---|---|
| All Mice CC5 - Brown | n=13/46 28% | n=7/52 13% |
| All Mice CC1 - Yellow | n=2/46 4% | n=7/52 13% |

❖ We investigated the effect of LDIR on the methylation status of the DMRs regulating imprinting of *Nnat*, *Peg3*, and *Igf2/H19* in male $A^{vy}$/a mice. We chose these genes because the inherited DMRs are known, and these imprinted genes play a critical role in metabolism, neurodevelopment, cognition, and behavioral formation (3-5); they are also dysregulated in cancer (6-8).

We demonstrated for the first time that exposure to LDIR significantly decreased the average DNA methylation of the DMRs that control genomic imprinting of *Peg3*, and *Igf2/H19* in male $A^{vy}$/a offspring by 5 and 3 percent, respectively (Figure 1), while the methylation status of the *Nnat* DMR was unaltered. The effects of LDIR on DNA methylation of *Peg3* and *Igf2/H19* is opposite of that previously observed at the *Agouti* locus (1). Thus, LDIR results in both increased and decreased methylation of gene regulatory elements; however, the effect of these alterations in DMR methylation on gene transcription and phenotype are presently unknown. A manuscript describing these results in detail is in preparation.

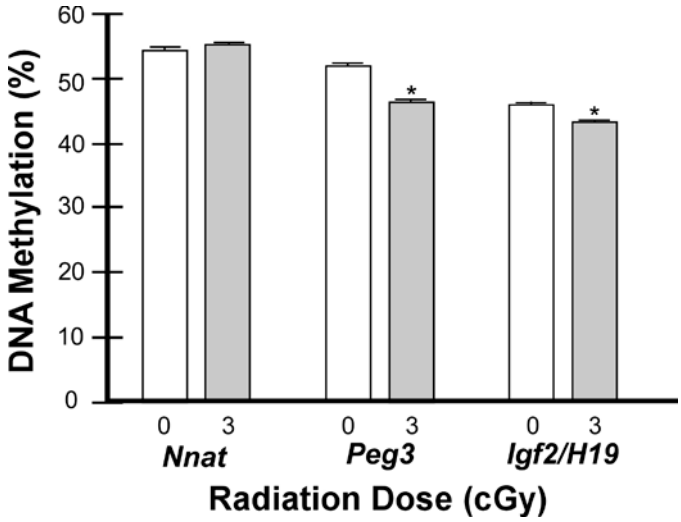


**Figure 1:** Average methylation at the differentially methylated regions (DMRs) that regulate imprinted expression of *Nnat*, *Peg3* and *Igf2/H19* in the liver of male $A^{vy}$/a mice. Percent DNA methylation was the average of CpG sites in the DMRs (*Nnat*, 4 CpG sites; *Peg3,* 12 CpG sites; and *Ifg2/H19*, 4 CpG sites). Error bars, SEM; (*, $p<0.01$)

## REFERENCES


1. Bernal, A. J., Dolinoy, D. C., Huang, D., Skaar, D. A., Weinhouse, C., and Jirtle, R. L. (2013) Adaptive radiation-induced epigenetic alterations mitigated by antioxidants. *FASEB J* **27**, 665-671
2. Dolinoy, D. C., Weidman, J. R., Waterland, R. A., and Jirtle, R. L. (2006) Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect* **114**, 567-572
3. Chen, D. Y., Stern, S. A., Garcia-Osta, A., Saunier-Rebori, B., Pollonini, G., Bambah-Mukku, D., Blitzer, R. D., and Alberini, C. M. (2011) A critical role for IGF-II in memory consolidation and enhancement. *Nature* **469**, 491-497
4. Li, X., Thomason, P. A., Withers, D. J., and Scott, J. (2010) Bio-informatics analysis of a gene co-expression module in adipose tissue containing the diet-responsive gene Nnat. *BMC Syst Biol* **4**, 175
5. Li, L., Keverne, E. B., Aparicio, S. A., Ishino, F., Barton, S. C., and Surani, M. A. (1999) Regulation of maternal behavior and offspring growth by paternally expressed Peg3. *Science* **284**, 330-333
6. Revill, K., Dudley, K. J., Clayton, R. N., McNicol, A. M., and Farrell, W. E. (2009) Loss of neuronatin expression is associated with promoter hypermethylation in pituitary adenoma. *Endocr Relat Cancer* **16**, 537-548
7. Gloss, B. S., Patterson, K. I., Barton, C. A., Gonzalez, M., Scurry, J. P., Hacker, N. F., Sutherland, R. L., O'Brien, P. M., and Clark, S. J. (2012) Integrative genome-wide expression and promoter DNA methylation profiling identifies a potential novel panel of ovarian cancer epigenetic biomarkers. *Cancer Lett* **318**, 76-85
8. Kaneda, A., and Feinberg, A. P. (2005) Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk. *Cancer Res* **65**, 11236-11240

**GRADUATE STUDENTS TRAINED**

1. Dana C. Dolinoy, Ph.D. – Duke University (Toxicology)
2. Autumn J. Bernal, Ph.D. – Duke University (Toxicology)

**POST DOCTORAL FELLOW TRAINED**

1. David A. Skaar, Ph.D. -- Duke University (Department of Radiation Oncology)

**PUBLICATIONS**


1. Skaar, D. A., Li, Y., Bernal, A. J., Hoyo, C., Murphy, S. K., and Jirtle, R. L. (2012) The human imprintome: regulatory mechanisms, methods of ascertainment, and roles in disease susceptibility. *ILAR J* **53**, 341-358
2. Bernal, A. J., Dolinoy, D. C., Huang, D., Skaar, D. A., Weinhouse, C., and Jirtle, R. L. (2013) Adaptive radiation-induced epigenetic alterations mitigated by antioxidants. *FASEB J* **27**, 665-671
3. Jirtle, R. L. (2014) The Agouti mouse: a biosensor for environmental epigenomics studies investigating the developmental origins of health and disease. *Epigenomics* **6**, 447-450
4. Soubry, A., Hoyo, C., Jirtle, R. L., and Murphy, S. K. (2014) A paternal environmental legacy: Evidence for epigenetic inheritance through the male germ line. *Bioessays*
5. Li, Y., Xie, C., Murphy, S. K., Skaar, D., Nye, M., Vidal, A. C., Cecil, K. M., Dietrich, K. N., Puga, A., Jirtle, R. L., and Hoyo, C. (2015) Lead exposure during early human development and DNA methylation of imprinted gene regulatory elements in adulthood. *Environ Health Perspect*